CLINICAL AND IMMUNOLOGIC ASPECTS OF FUNGOUS DISEASES

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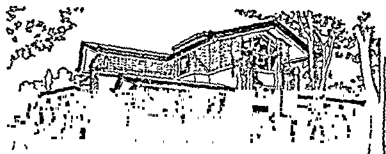
CLINICAL AND IMMUNOLOGIC ASPECTS OF FUNGOUS DISEASES

By

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INTRODUCTION

There are several excellent textbooks of medical mycology, presenting the subject in a well balanced form. The field is so large, however, that space limitations cause some aspects to be discussed only briefly, while some even more potentially valuable material is omitted entirely because it is controversial or insufficiently well understood for the implications to be covered with sufficient brevity.

The present volume is not intended to compete with such complete texts, but to supplement them somewhat by presenting the clinical and immunologic aspects of fungous diseases in more extended detail. While it is primarily intended for clinicians, it should appeal also to all who are interestd in medical mycology and the study of infectious diseases in general beyond the level of pure laboratory technology. In particular it is hoped that some of the deficiencies in our factual knowledge in this field will be eliminated by research directed against some of the problems spotlighted by this discussion.

Being a clinical physician in private practice, and non-salaried in any research or teaching capacity, it is far beyond this author's time or intellectual capabilities to present this subject in a form deserving to be entitled "The Pathogenesis of Fungous Disease" as did Rich so admirably in the field of tuberculosis. It is hoped, however, that someone of such caliber will read this volume, and be stimulated sufficiently by its shortcomings to produce an adequate treatise

To the casual observer, the sequence in which the various diseases are treated here will seem entirely purposeless, and the proportion devoted to certain disorders grossly overbalanced. The author, however, believes that this format will convey the maximum in clarity of understanding to those who will read continuously from cover to cover. Realizing nevertheless that not everyone can or will do so, and that inevitably there will be times when but one of its chapters will be consulted as a hasty reference by someone interested in only a single disease entity, an attempt has been made to cause each chapter to be intelligible if read alone, at least if its references to other pages are followed. There is thus some duplication in the various chapters, for which the patience of the *ideal* reader is solicited.

Fungi cause disease in human beings in two widely divergent types to which the adjectives "superficial" and "deep" are usually applied. The former is acquired with extreme frequency, probably by the great majority of all persons at some time or other, but only rarely in a form sufficiently severe to be classed as more than a nuisance. Here the fungi limit their pathogenic activities to the skin, being apparently unable to survive in any of the deeper tissues of the body. In fact, their range is usually even narrower, involving only the dry, keratinized portions of the skin, hair and nails, producing no lesions which could cause a permanent mark to be left upon the body, and inducing no demonstrable changes in its living cells, or in its immunologic or allergic capabilities.

In a small percentage of cases, however, the infection penetrates more deeply, even incapacitating the individual for a considerable time and producing changes which are sometimes permanent in his immunoallergic status. In spite of extensive study, the various factors which influ-

ence these processes, and to what extent they may progress, are not yet well understood, resulting in embarrassing limitations in the effectiveness of diagnostic and therapeutic methods. It appears probable that progress in this field might be accelerated by applying principles learned by *first* studying other infectious diseases, in some of which changes apparently of corresponding significance are to be encountered, but in much more pronounced degrees. It is believed that some of the "deep" mycoses in particular have much to offer in this regard. Thus, in the present volume, the superficial fungous infections are discussed last, not because they are relegated there as being unimportant, but because it is thought that more can be accomplished by first establishing a base by studying more easily understood diseases

Because of the comparative rarity with which they are acquired by human beings, the *systemic* fungous infections have received but scant attention by the vast majority of scientists. While it must be admitted that, at the present time and under ordinary circumstances, seldom will an opportunity arise for any individual practitioner to utilize knowledge of such diseases directly to the benefit of a patient, there are several reasons why an intimate study of this subject is to be highly recommended

First, there is such a wide variation in the clinical pictures produced by the several "deep" mycotic infections, that they must be constantly kept in mind in the differential diagnosis of any syndrome whose etiology is not at once otherwise clearly evident For lack of wider dissemination of this knowledge many such cases have been, are being at present, and will continue to be missed completely; or diagnosed only in the last stages, too late for any attempts at therapy to be successful, and frequently

not until necropsy, furnishing then a case report lacking many of the features of the clinical course of the disease necessary to enable it to be studied in retrospect and to be included subsequently in any statistical investigations. Progress is thus greatly retarded, since the very rarity of these cases makes it difficult for any one physician to become expert, from his own personal experience, limited as it must almost certainly be to the observation of only a few such instances within his lifetime.

Second, there is reason to believe that systemic fungous infections have been becoming more prevalent recently and that they will probably continue to increase in incidence. In some instances areas of endemicity appear to be enlarging and new foci are being discovered at some distance from the original locus indicating a need for better epidemiologic understanding. Furthermore, as chemotherapy and antibiotics reduce the pathogenic activities of bacteria and spirochetes, fungi have an enhanced opportunity; some such drugs seem even to promote the likelihood of infection by these organisms. As other fatal diseases become better controlled, the incidence of systemic fungous infections will increase in percentage *if for no other reason than the fact that we must* all eventually die from some cause or other.

Third, (and by far the most important) there are in*dications that our knowledge of infectious diseases in* general could be greatly increased by utilizing pathways blazed by first studying the deep mycoses, in which *certain features are much less complex* than in non mycotic infections

With regard to the therapy of fungous diseases them selves, it is becoming increasingly apparent that the attainment of complete success through the discovery of a perfect chemical fungicide is unlikely To be ideal such

a substance should have many attributes; it should be stable, soluble, colorless, odorless, tasteless, absolutely nontoxic to human beings, free of undesirable side-effects, and active against all pathogenic fungi wherever they might be located in the body, and in concentrations easily attained and maintained by oral or parenteral administration. To reach such a goal would indeed require a constellation of fortuitous circumstances far beyond the anticipation of any serious scientist. Yet an astounding amount of effort has been expended in this direction, principally because the possible financial rewards loom so large In itself this effort is not entirely regrettable, since it may succeed eventually, in part at least. But it is alarming to observe how little in comparison is being done in other directions.

It is profoundly significant that no fungous disease reaches serious proportions or lasts for any considerable time unless it involves a person who is abnormal. It will be emphasized in the succeeding chapters that, for example, coccidioidomycosis becomes serious in only one of every thousand persons who acquire the disease, and that that one person must possess a defect in his immunologic processes; in fact this defect almost certainly was present even before the infection was acquired. As another example, of one hundred persons infected with *Trichophyton rubrum* continuously for many years, there will be not more than one spouse who becomes infected, even though living in an environmental sea of viable spores, while there will be a few children (blood relatives of the patients) who do become involved. Here there must be a defect of some sort in resistance which allows infection to take place, and this defect seems to be hereditarily transferable.

Because of many such phenomena, it is unwise to channel all of our effort into studying the fungi and their chemical adversaries; we need to study equally diligently the mechanisms of natural and acquired immunity and resistance against their invasion. We need to study the *few* patients who have these diseases in *serious* form and learn wherein they are deficient and how the deficiency can be corrected. It is probable that such a return to normal in this regard would find the patient able to fight the infection successfully without benefit of fungicides.

In other words, too much effort is being expended in studying the *bugs* and the *drugs*, and not enough in studying the *mugs*, the *lugs*, the *pugs* and perhaps the *thugs* who have *acquired* these infections.

In this regard, the author believes that the systemic fungous disease coccidioidomycosis can be an especially valuable guide in the study of infectious diseases. This disorder exhibits a unique series of well defined phenomena in the realms of epidemiology, pathology, allergy and immunology which can be apparently correlated with the clinical course of the disease in such a manner as to furnish better criteria for prognosis and treatment than we have in other diseases. Some of these phenomena appear to be duplicated, at least partially, in several other deep mycoses, and indeed in some non myotic diseases, so that by using experience gained in the study of coccidioidomycosis, one can sometimes pursue an interesting thread of theory through a maze of apparent contradiction In attempting to follow a few such pathways the author has encountered several instances in which certain hither to perplexing aspects of infectious diseases seem to be rendered logically explainable, indicating that perhaps the trail is indeed the right one

Beginning slowly and continuing admittedly intermittently at first the author has been a student of coccidioidomycosis in an ever increasing degree for some 25 years. During this interval he has had the benefit of personal acquaintance with and the teaching efforts of many of the pioneers responsible for our present factual knowledge of that disease, including Howard Morrow, Hiram E. Miller, Harry P. Jacobson, John F. Kessel, Myrnie A. Gifford, Charles E. Smith, Robert M. Stewart and Edward M. Butt. It is admitted at the outset that any one of these, his teachers, has probably had a richer actual observational experience in this field than he, and that he therefore is subject to criticism for his presumption in allowing himself to be selected as the proper one to prepare this monograph, since it were better done by one more expert.

In partial expiation of such presumptiveness, however, it can be pointed out that the author has had several other peculiarly advantageous opportunities to study fungous infections. The greatest of these has been an almost brotherly alliance during the past 12 years with a mycologist, exceptionally well grounded botanically, and greatly interested in the medical aspects of that specialty, Doctor Orda A. Plunkett of the University of California at Los Angeles. This relationship has furnished a foundation in mycology firm enough to support a stature much greater than the author can ever hope to attain. In addition, for several years he has been privileged to be one of a group of scientists assembled yearly for the purpose of teaching medical mycology to members of the American Academy of Dermatology and Syphilology. An opportunity has been thus afforded to keep his views concerning the deep mycoses concurrent with the latest facts by intimate discussions with those especially experienced in that field. J Gardner Hopkins, Fred D Weidman, George M.

Lewis, Arthur C. Curtis, John H. Lamb, Norman F. Conant, Lucille K. Georg, Mary Ellen Hopper, J. Lamar Callaway, John A. Gammel, Jacob H. Schwartz, Arturo L. Carrion and Leslie M. Smith have thus contributed to his education, for which he is profoundly grateful. Personal interviews with Chester W. Emmons of Washington, M. L. Furculow of Kansas City, Floriano P. de Almeida and Carlos da Silva Lacaz of São Paulo, Brazil, Flavio L. Niño and Pablo Negroni of Buenos Aires, Arêa Leão of Rio de Janeiro, Juan E. MacKinnon of Montevideo and Antonio Gonzalez Ochoa of Mexico have been especially valuable.

Also, the author is perhaps the only physician fortunate enough to have had the opportunity to observe and follow *three* cases in which organisms capable of causing systemic fungous infections were *known* to have been inoculated intracutaneously into human beings. The study of these almost "experimental" infections afforded clues which clarified some perplexing aspects of his views.

It has long been obvious that all of the experienced scientists heretofore mentioned have followed and will undoubtedly continue to follow the ethics and conventions of scientific writing (even as has, indeed, this author until now); and that therefore that portion of their views which exists in their minds as mere theory and concept will be withheld from publication *until it* can be supported by adequate proof. Such proof will not easily be assembled *by any one investigator if* he waits until he has personally observed a sufficient number of cases of disease as rarely encountered as the systemic fungous infections. The author believes that such conservative waiting has already caused some contributions invaluable to the clarity of our understanding of these diseases to be lost to us by the death of their possessors

Hence, in these introductory paragraphs of this monograph it is necessary to warn the reader specifically to avoid the inference that there is as yet adequate proof for all the views expressed herein. Wherever exact references cannot be cited it is beyond the power of the author to recall which of these ideas originated within his own mind and which were implanted there by conversations with other persons or by reading. No claim is made, therefore, for originality in *any* of these theories; they have simply been collected so that they could be presented together, supported by facts wherever possible and by argument where proof is not yet at hand. It is not anticipated that all of these concepts will eventually prove to be valid; indeed it is virtually certain that some will not. It is considered safe to proceed, however, since every effort has been made to insure that the presentation of these views will not cause harm to come to any patient but will instead tend to add to the efficiency with which his disorder may be combatted.

It is hoped that four purposes may be accomplished:

First, that the study of fungous diseases in general will be stimulated, resulting in the correct diagnosis of a larger percentage of those cases actually in existence, and early enough in the course of the disease to afford time for a therapeutic approach to be successful.

Second, to focus attention upon the value of more detailed investigation and recording of the facts concerning each individual case of entities so rare as are some of these, so that the resulting case reports may be studied subsequently by all interested scientists without discovering omissions rendering them useless for statistical inclusion.

Third, to furnish improvement in our guides to prognosis and therapy in the mycoses and perhaps in other infectious diseases, thereby (1) guarding against relapses

due to injudiciously early resumption of physical activity, (2) avoiding unnecessary and sometimes harmful medical and surgical intervention, and (3) rendering the evaluation of drugs and other therapeutic modalities much more accurate.

Fourth, to invite the cooperation of all workers in the critical evaluation of these concepts, so that they may be proved or disproved, (and whenever possible *improved*), leading to a true understanding of the pathogenesis of these diseases and to their therapeutic conquest before the lapse of the several decades which might otherwise be required.

CONTENTS

CLINICAL AND IMMUNOLOGIC ASPECTS OF FUNGOUS DISEASES

Chapter 1

COCCIDIOIDOMYCOSIS AS A GUIDE IN THE STUDY OF INFECTIOUS DISEASES—THE EVOLUTION OF THE CLINICAL CONCEPTS

IN SPITE of intensive research extending over many years the processes by which the human body acquires immunologic resistance to most of the chronic infectious diseases remain obscure. With few exceptions, methods by which such resistance may be artifically produced or stimulated have not been discovered.

Many features of the pathogenesis and immunology of coccidioidomycosis are apparently much less complex than those encountered in other infections Because of the conviction that intensified study of this disease might yield clues leading to a better understanding of other more complicated infections, it seems worth while to present here the subject of coccidioidomycosis in considerable detail, attempting thereby to establish a foundation of understanding which will serve to support later discussions of more difficult problems. A brief factual background of the clinical and pathological aspects is included, but the details of the mycologic laboratory procedures are omitted since they are thoroughly covered in many other texts.

Gifford examined stools, and sputa from such patients. She found no worms, but a fungus was repeatedly observed, and when culturally studied was identified as *Coccidioides immitis.* Discussion of this phenomenon with Dickson, who had observed a case of coccidioidomycosis originating as a laboratory infection by the inhalation of material from an old, dry culture, led to further studies, and it was not long before this fungus became firmly established as the sole cause of "Valley Fever," entering the body by inhalation of spores borne with dust upon the wind. Thus there was brought to light another form of cocciodioidomycosis, which was entirely different from the chronic granulomatous, widespread highly fatal type previously so well recognized and which, at the time of its first discovery, bore no *apparent* relation to it. In contrast, in *these* patients the infection remained limited to the lungs, caused temporary allergic reactions in the skin, and resulted almost invariably in complete recovery without sequelae. This became known as "primary pulmonary coccidioidomycosis" and is synonomous with "Valley Fever."

However, it was soon noted that a few patients who had been diagnosed as having primary pulmonary coccidioidomycosis did *not* improve after a short time, as was the rule, but instead grew worse. Lesions eventually appeared in the skin, bones and viscera and the clinical picture gradually changed to that of the long familiar coccidioidal granuloma It was soon proved that this syndrome developed because the organisms had been scattered from the primarily infected lungs extensively throughout the body by way of the blood stream. Subsequently the term "disseminated coccidioidomycosis" began to supersede its synonym "coccidioidal granuloma"

Following a small but most instructive epidemic in which 10 of 14 persons developed "Valley Fever" following the inhalation of desert dust raised by an attempt to dig a rattlesnake from its hole, Stewart and Meyer were successful in culturing *Coccidioides immitis* from the soil of the area, a finding confirmed many times since. It now became clear that the epidemiology of the infection was for the most part dependent upon climatic conditions favorable for the production and geographical dissemination of terrestrial dust. Emmons pointed out that certain rodents may serve as vectors to infect new areas of soil.

During the latter portion of the "early period" investigations began to be carried out dealing with the immunologic and allergic manifestations of coccidioidomycosis, and many important discoveries have since been made. These features are to be presented in detail in succeeding chapters where their tremendous significance can be pointed out.

In what may be called the "recent period" another clinical form of coccidioidomycosis has been delineated. It will be recalled that the early workers believed that "coccidioidal granuloma" originated sometimes as the result of direct primary inoculation of the causative fungi into the skin by means of a puncture wound. With the discovery that "Valley Fever" was actually "primary pulmonary coccidioidomycosis" and that *all* of the previously observed forms of the granulomatous type could result by dissemination of the organism from that focus through the circulating blood, fewer and fewer cases were subsequently attributed to *direct cutaneous* inoculation. Nevertheless some cases were still so accepted until in 1947 this author, Smith and Plunkett were privileged to study a patient who was *known* to have received an inoculation of *Coccidioides immitis* into a finger wound. The resulting syndrome did

Primary Pulmonary Coccidioidomycosis

Far from being the rare disease it was originally considered to be, it is now established that almost all previously uninfected persons who inhale *C. immitis* in any significant quantity acquire the disease. The incubation period is from 10 days to 2 weeks. In more than half of these persons, however, the infection produces no symptoms whatever, and they remain entirely unaware of its presence throughout its course, after which they are nevertheless apparently immune to reinfection. These facts are revealed only because during the process these individuals acquire the ability to react to the "skin test" with coccidioidin (to be discussed in detail later in Chapter 6). This test reveals an increasing percentage of positive reactors among those "normal" persons born into or moving into endemic regions for each year of their subsequent residence there, in some regions reaching 90% or more within 3 or 4 years. Even in those cases in which symptoms *do* appear, they are frequently very mild, being classed by both patients and physicians simply as "colds" or "influenza." However, in others the illness may be very severe, accompanied by pulmonary symptoms and signs of almost any type and degree, none of which, however, is specific for this disease. Fever, malaise, chills, cough, noctidrosis, nasopharyngitis, chest pain, headache and backache are commonly encountered.

Pathologic pulmonary changes occur in a wide variety, including various combinations of pleurisy, effusion, hilar thickening, parenchymal nodules, bronchopneumonia or lobar consolidation, cavity formation and miliary scattering. Physical examination usually reveals less than the changes which are actually present would indicate. Rales, dullness, suppression of breath sounds

and pleuritic friction rubs may be demonstrable. X-ray examination is frequently negative even in well developed cases, but in others a wide variety of abnormalities may be encountered most of which cannot be differentiated from those due to other pulmonary diseases. A fuzzy peribronchial infiltration in the hilar region is the rule in mild cases, while in more severe illness a soft, homogenous bronchopneumonic infiltration is characteristic, more likely to favor the middle and lower lobes in contrast to tuberculosis. In these areas there are often isolated, well circumscribed nodules, suggestive of metastatic malignancy. Cavities occur frequently, characterized by being exceptionally thin-walled and free of surrounding parenchynial infiltration, so as to suggest cysts, they are frequently not revealed by ordinary flat radiographs but only by planograms. Plural effusion occurs in about 20% of cases but tends to be minimal and evanescent.

In from 1 to 2 weeks after the onset of symptoms, allergic eruptions appear in percentages of cases varying from 30% in white women to 2% in deeply pigmented men. Any form of erythema multiforme may be seen, but the commonest and most typical is erythema nodosum, consisting of one or a few large tender, erythematous nodular swellings situated usually below the knees. These manifestations are caused *not* by the spread of the *infection* itself, but by the hematogenous scattering of *toxic* products derived from the *pulmonary* focus, acting in conjunction with the specific cutaneous *hypersensitization* which has developed, manifested in parallel fashion by the ability to react to the skin test with coccidioidin There are, therefore, "coccidioidids," analagous to trichophytids, tuberculids, etc. Acute arthritis occurs occasionally by the same mechanism. Any of these allergic reactions is usually accompanied by a significant degree of

eosinophilia. This reactivity is extremely valuable in prognosis, because patients who exhibit any of these phenomena are virtually certain to recover from the disease rapidly and completely; the significance of this important fact will be discussed later in Chapter 6.

Primary pulmonary coccidioidomycosis results in complete recovery in all but a very small percentage of cases, approximately one per thousand, and there are no sequelae except the persistence of the ability to react to the skin test with coccidioidin and an apparently complete immunity to reinfection which endures for many years, probably for life. Recovery may be delayed for some months, however, by extensive pneumonic infiltration, parenchymatous nodules or large cavities. Such patients must be carefully guarded, lest they resume physical activity prematurely and thereby lose the chance of complete recovery. They should also be watched carefully for evidence of dissemination.

Primary Cutaneous Coccidioidomycosis

As has been stated, it was formerly believed that coccidioidomycosis occurred frequently by direct inoculation of the fungus into the skin, but recent events indicate that it is probably extremely rare It must be admitted that a lesion in the skin is indeed frequently the first indication of the presence of the disease, but in almost all recorded cases the subsequent course of events strongly indicates that the organisms were *brought* to that point by being disseminated from a *primary pulmonary focus, previously unrecognized* because of being too mild to attract attention. This is no longer difficult to accept since it has long ago become evident that more than half of the cases of the primary pulmonary type *remain entirely subclinical*, and

many others are never recognized specifically as coccidioidal infections.

This subject might have remained obscure except for a most instructive case observed in 1918 by Wilson, Smith and Plunkett, that of an embalmer who acquired an infection in an abrasion on his finger while preparing the body of a person dead of disseminated coccidioidomycosis. An indurated, relatively painless ulcerated lesion appeared at the inoculated site, closely resembling the primary "chancre" of syphilis or of sporotrichosis. There was mild fever. In a short time eight nodules appeared along the lymphatic channels draining this area accompanied by lymphangitis, followed by epitrochlear and axillary adenopathy. The picture was identical with that of the usual type of *sporotrichosis.* Without the benefit of any treatment which could have been considered specifically effective, the entire syndrome subsided and the patient has remained well up to the time of this writing

These authors enumerated several criteria which they believe should be fulfilled if a case is to be acceptable as of this type. In the order of their chronological appearance, these are as follows

Criteria for a Cutaneous Portal of Entry

1. There should be no history of significant pulmonary disease immediately preceding the appearance of the cutaneous lesion

2. The history should be suggestive of inoculation through a break in the skin at the site of the first cutaneous lesion observed. Simple "injury" such as a bump or bruise should be considered insufficient evidence

3. Only a short incubation period should elapse, probably between one and three weeks, before a visible cutaneous lesion develops

4. The primary lesion should resemble a "chancre" as seen in primary syphilis or the primary cutaneous tuberculous complex, rather than an abscess or torpid cutaneous ulcer. The lesion should be a relatively painless, firmly indurated nodule or nodular plaque, with central ulceration.

5. The precipitin reaction to coccidioidin* should soon become positive. It should decline somewhat more slowly than if the disease had been present in the lungs for a few antecedent weeks.

6. The response to the intracutaneous injection of coccidioidin* should become positive and should increase in sensitivity (1-1000 dilution) unless immunity fails to develop.

7. The complement-fixation reaction* should be negative at first, and remain so for several weeks, after which it should be present only in low titer, unless immunity fails to develop.

8. Lymphangitis and lymphadenopathy should develop, but in the region of drainage only. Development of nodules similar to those seen in sporotrichosis may be expected.

9. Spontaneous healing of the "primary" cutaneous syndrome should occur within a few weeks (unless the patient is immunologically defective: This should be anticipated in but one or two per thousand instances).

A careful search of the literature up to the time this paper was published indicated that there had been only one similar case previously reported (Guy and Jacob). Very recently, however, Trimble and Doucette have studied a coccidioidal infection acquired percutaneously by a laboratory worker whose skin became contaminated by culture material of *Coccidioides* The clinical picture

*See Chapters 3 and 4

was identical to that of the patient of Wilson, Smith and Plunkett.

These three cases were entirely different from all others *presumed* to have been primarily inoculated into the skin. In contrast, all of these others presented lesions entirely in keeping with those commonly observed in the disseminated disease. Most of them in fact "*progressed*" to the disseminated form, which indeed they almost certainly were already when first observed.

Almost all persons who acquire coccidioidomycosis by the primary *pulmonary* route recover quickly and are thereafter immune to reinfection. Only one or two per thousand, immunologically defective, subsequently have the disease in the disseminated, granulomatous form that often causes death. It would be most unlikely that the disease caused by primary cutaneous inoculation of the organisms would pursue a different course; indeed, since the skin is so highly reactive to the products of the fungus (coccidioidin) in those persons who do acquire immunologic resistance, inoculation by the intracutaneous route theoretically should result in conferring immunity even more quickly and consistently than does pulmonary infection Since rapid involution of the primary cutaneous chancre and its sequelae should be expected in all but one or two per thousand instances, the continuation of a cutaneous lesion for more than three months should be considered strong evidence against its having arisen by primary cutaneous inoculation

Disseminated Coccidioidomycosis, Coccidioidal Granuloma

Of patients possessing primary pulmonary coccidioidomycosis a small percentage, about one in a thousand, fails to fight the disease immunologically, acquires more

and more extensive pulmonary involvement, and finally begins to develop lesions by the hematogenous dissemination of the *living organisms* to the skin, bones, central nervous system or other viscera. In striking contrast to the benign primary form, this type is fatal in perhaps 50% of instances.

Although the inhalation originally of massive amounts of fungus spores cannot be excluded as a significant factor, the principal cause of dissemination seems to be the fact that certain individuals apparently possess an immunity mechanism inherently defective in some vital feature. Whether or not dissemination is destined to take place is apparently determined very early in the course of the infection, probably by an immunologic deficiency present even *before* the inoculation occurred. For reasons as yet not understood, dissemination is more common in males, and in those whose skins are deeply pigmented, and it is this fact, of course, which renders the prognosis so much *poorer in such individuals.*

In coccidioidomycosis we are not as yet able to discern the nature of this postulated "inherent defect" in the immunity mechanisms As will be shown later (see page 158 and page 178) in histoplasmosis and cryptococcosis there is a statistically significant relationship between serious forms of the infections and diseases of the reticuloendothelial system, suggesting that such diseases render this system deficient in the performance of one of the most important duties attributed to it, that of producing antibodies by which specific immunity against a certain infection is conferred. No such correlation has been thus far observed in coccidioidomycosis.

Beginning in 1954, L. Pillemer and a group of coworkers centered about the Institute of Pathology at Western Reserve University have presented a series of re-

ports concerning intricate investigations revealing that normal serum possesses a protein component which is apparently intimately concerned with the ability of animals to resist infections. This substance, named "properdin," appears to be nonspecific as regards any one infection. In the presence of the third component of complement and magnesium ions it can combine in such a way as to kill or inactivate certain bacteria and viruses and participate in the lysis of abnormal red cells. At the time of this writing no observations with regard to fungous infections have been reported, and it is not possible to analyze the possibilities in relation to host resistance to *Coccidioides*. At first glance, this mechanism seems unlikely to be implicated in the development of the highly specific form of immunity so prevalent following coccidioidal infections, but it might nevertheless be shown that those few persons who fail to develop this immunity were deficient in properdin during the initial invasion, and because of the lack of this "natural immunity" factor, the infection progressed so rapidly as to overwhelm the power of developing "specific" resistance. Properdin can be readily assayed in serum by using certain high-molecular polysaccharides (zymosan), and it should be possible to determine whether a deficiency in this component is demonstrable early in the course of coccidioidal infections in those persons who later sustain dissemination. In this event it seems possible that purified properdin might be made available to carry the patient over the critical period. Under certain circumstances the blood level of properdin has been raised by artificial stimulation, but thus far only after an initial depression which might more than outweigh the advantage of the later rise.

Dissemination may occur early in the course of the disease and proceed rapidly to massive involvement by

widely distributed tiny foci simulating miliary tuberculosis, and leading to early death. At the other extreme a person previously entirely unaware of any illness may develop a solitary lesion which then progresses *slowly* or even *intermittently*. Many such lesions may heal and some of these patients eventually recover completely. In others still more foci develop, enlarge and ultimately cause death sometimes after only a few months, sometimes not for years.

Dissemination most commonly involves the lungs, skin and subcutaneous tissues, bones, joints viscera, meninges and brain. Except in those very rare instances in which the skin furnished the *portal* of *entry*, the discovery of extra-pulmonary lesions is proof that *dissemination* has occurred. It is in striking contrast with tuberculosis to observe that the gastrointestinal tract is remarkably resisant to coccidioidal involvement, resisting the organisms when they are disseminated to it by the blood stream as well as when they are ingested.

Dissemination is to be *suspected* in the lungs themselves, rather than simple increase in severity of the primary infection, when the mediastinal adenopathy is marked and progressive, and when lesions resembling the *adult* type of tuberculosis develop, particularly in the apices. *Disseminated lesions in bone are likely to select* points of tension or pressure such as the insertion of tendons or ligaments; they present a peculiar "punched out" appearance, resembling cysts, with a sharp line of *transition from destroyed bone to that which is still* healthy. If bony lesions extend to involve joint cavities the weight bearing surfaces are characteristically spared at first in contrast to tuberculosis

Dissemination into the skin produces one or more subcutaneous abscesses which slowly enlarge, and after

spontaneous rupture or incision leave chronic indolent draining sinuses. At the periphery of such lesions the skin frequently becomes extensively secondarily infected, simulating scrofuloderma. Occasionally large areas may ultimately be involved in this manner in an ulcerative, granulomatous process exhibiting irregular serpiginous borders and central atrophic scars, bearing considerable resemblance to chronic cutaneous North American blastomycosis.

In the viscera, lesions usually do not appear in a single organ, but tend to be more widely disseminated. Involvement of the central nervous system is more common in light skinned persons and is usually in the form of meningitis; it is almost always fatal, although sometime slow to progress

In all types of disseminated coccidioidomycosis fever, chills, prostration, and progressive emaciation are typical symptoms. A hypochromic anemia is commonly found. The leucocyte count is variable, and the erythrocyte sedimentation rate usually remains high

Pathology

The pathologic response in the tissues during the course of disseminated coccidioidomycosis is an unusual feature. When a mature spherule ruptures and discharges endospores an acute inflammatory response is induced in the immediate area with the collection of myriads of polymorphonuclear leukocytes--a suppurative reaction. As each endospore enlarges, the reaction around it gradually becomes more chronic and lymphocytes replace the polymorphonuclear cells, then macrophages appear together with plasma cells and large mononuclear cells Still more growth of the spherule produces an ever more chronic infiltrate until epithelioid cells predominate, finally assum-

ing a tuberculoid structure with giant cells in the center where the mature organism is found with its endospores When the spherule ruptures the entire cycle is repeated. Thus, the general appearance microscopically is that of a tissue reaction of mixed type, varying from the most acute in one tiny area to the most chronic in another close by. When to the series just described is added the eosinophilic infiltrate so characteristic of the allergic erythema multiforme stage of primary coccidioidomycosis, it is realized that this disease runs the entire gamut of non-neoplastic pathologic infiltrations.

In histopathologic study of material taken from the original "chancre" in the case of primary cutaneous coccidioidomycosis reported by Wilson, Smith and Plunkett, a similar reaction of mixed type was noted, varying from an acute inflammatory infiltrate in some areas in which polymorphonuclear cells predominated with some eosinophils and lymphocytes, to a chronic, granulomatous reaction in contiguous spots, in which epithelioid cells, small lymphocytes and giant cells were seen. With the exception of the eosinophils, these are the histopathologic features commonly considered *to be typical of coccidioidomycosis*, regardless of the location or type of infection. The coccidioidal spherules were sparsely distributed and difficult to discover. The same pattern was observed in material taken from the regional lymph nodes.

These features are at variance with those to be expected in primary "chancres" of other infectious granulomatous diseases, at least in the early phases wherein an acute reaction predominates and the causative organisms are present in large numbers. It is most important, however, to recall that the tissue examined *in the case in question* was not obtained until five weeks after the onset of the infection, at a time when considerable involution had

taken place. In comparison, a "chancre" due to syphilis would have similarly involuted during such an interval so that the original acute phase of the inflammatory process would have become more chronic and the spirochetes much reduced in number. Hence it does not seem warranted to conclude that the histopathologic studies in this case supplied evidence against an intracutaneous portal of entry, although it must be admitted that they did not assist in proving it.

This question has now been solved by the observations of Trimble and Doucette, who were privileged to obtain specimens for histopathologic study very early in the course of the disease in their patient. They reported that the early phase was characterized by an acute inflammatory reaction within which fungous spherules were numerous and easily seen. This is consistent with the picture to be expected in the primary "chancriform" stage of other diseases.

Diagnosis

The diagnosis of coccidioidomycosis is made by discovering the typical spherules of *Coccidioides* containing endospores, in sections of diseased tissues or by recovering the fungus in artificial culture or by animal inoculation. Details of these precedures are available in textbooks of mycology. It is also accepted that a patient who exhibits no reactivity to the skin test early in the course of an illness, but develops positive reactivity later can be accepted as having the disease. A positive reaction to the specific complement fixation test in more than small, doubtful concentrations indicates the presence of active coccidioidomycosis, as does a positive reaction to the coccidioidin precipitin test

Chapter 3

COCCIDIOIDOMYCOSIS AS A GUIDE IN THE STUDY OF INFECTIOUS DISEASES—THE SIGNIFICANCE OF THE INTRACUTANEOUS COCCIDIOIDIN TEST

BEGINNING in 1915 several early investigators working with coccidioidomycosis, notably Cooke, Davis, and Jacobson, investigated skin testing procedures using extracts of cultures of *Coccidioides* which they called "coccidioidin." What would be considered by today's standards to be tremendous quantities (0.3 to 0.4 cc. of the undiluted extract) were injected *intracutaneously into patients known to be suffering from coccidioidomycosis and into a series* of "normal" controls. The delayed tuberculin type of response was often obtained, but it was concluded finally that the test had little specificity and was worthless for diagnostic purposes, since the majority of patients with *proved* coccidioidomycosis reacted minimally or not at all, while severely positive reactions were encountered frequently in the *"control"* group, that is in persons *who had* never been ill in a manner even remotely suggesting that *disease*. In the light of subsequent events this conclusion stands as a shining example of a testing procedure receiving the blame for shortcomings eventually proved to be attributable to those persons interpreting the significance

of the results; for the *test* was always *right*, it was the *observers'* interpretations which were *wrong*. They are not to be censured, however, since they were not aware of certain vital facts which have since been discovered.

However, as the disease itself began to be better understood, especially after Gifford and Dickson's discovery that "Valley Fever" is actually a milder form of coccidioidomycosis, these reactions to coccidioidin began to be clarified by many observers, by far the most important of which were in two groups, one headed by Charles Edward Smith, and the other by John F. Kessel.

It was inevitable that as soon as the coccidioidal nature of "Valley Fever" was recognized, such patients were tested intracutaneously with coccidioidin. Except in the first week or two of the illness, a positive reaction was found to be the rule; in fact many instances were observed illustrative of the manner in which the response actually *developed*, there being no reaction at the first testing, but a positive response to reapplication a few days later. This *"conversion"* from negative to positive during the *early phases* of an acute upper respiratory infection has now become accepted as *diagnostic* of its coccidioidal nature. Particularly significant were the results of such skin testing in those patients who showed erythema nodosum or multiforme as a complication, *all* of whom exhibited an astoundingly *high degree* of reactivity, necessitating dilution of the testing material to 1-1000 or 1-10,000, if unduly severe damage was to be avoided at the site of the injection, sometimes accompanied by an intense flareup of the multiform skin lesions. To emphasize the close relationship between coccidioidin and the "toxic substance" circulating in the blood which results in the allergic manifestations of erythema nodosum or multiforme, the *mere application* of the cutaneous test was occasionally observed

to actually precipitate such manifestations, or to cause them to *recur* after they had once subsided.

Carrying this program still further, the conversion *of the test from negative to positive* was observed during the course of many cases of respiratory infections occurring in seasons of the year other than those in which "Valley Fever" was to be expected or in general too mild to have been otherwise recognizable as such. Then it was shown that in certain *geographic areas a high percentage of persons in the general population* are able to react positively to the test, in spite of the fact that the majority of them can recall *no illness* whatsoever suggestive of the diesase: also that the percentage of positive reactors increases as the *age-group* of the individuals increases, and as the *length of residence* in the endemic area increases so that in some *regions four years of residence almost certainly* confers positive reactivity (rate of infection 25 to 50% per year.) That this infection is indeed limited to certain geographic areas was evident when no positive reactors to the test using the same material were found among the general population in several regions in the Eastern United States.

One aspect of the significance of the intracutaneous coccidioidin test now began to be clearly understood It became evident that the ability to react to this procedure is *acquired* during the course of an acute respiratory infection with *Coccidioides*, even though it is often *entirely subclinical;* and that this reactivity *persists*, perhaps for life, but at least for many years after the cure of the disease. A positive reaction thus indicates that the disease either *is present* at the time of the test, or *has been present* at some time *in the past life of the individual.* (These facts are, of course, consiste… exper… ith the tuberculin skin test.) Tl

ease, coccidioidomycosis is now known to be very *common,* and to be acquired by *almost all* exposed persons, causing a pulmonary disease of varying intensity but, in more than half of the cases, so mild that the individuals remain entirely *unaware* of its presence.

However, it was observed that a certain few patients suffering from an acute respiratory infection classifiable as coccidioidomycosis by the culture of the sputum *did not* develop the ability to react to the skin test with coccidioidin, or reacted but *poorly* and for a *short* period of time only. Some of these patients eventually developed the chronic, granulomatous, disseminated form of the disease and died without ever regaining any high degree of reactivity to the test. It soon became established that this cutaneous anergy toward coccidioidin is the *rule* in fatal cases, except when death occurs with such great rapidity, that it overwhelms the patient before sufficient time has elapsed for whatever degree of reactivity has been present to be entirely lost (e.g., in meningitis.) This terminal anergy is consistent also with the known facts concerning tuberculin testing. Thus a *negative* coccidioidin skin test does not *rule out* the presence of coccidioidomycosis; persons destined soon to *die* of the disease will usually be as equally non-reactive as those who have *never* had it at all.

The mechanism by which this "anergic" phase is produced is not clear. In *other* diseases such "terminal anergy" has been most frequently attributed to saturation of the receptor antibodies in the skin by excessive amounts of "circulating antigen" derived from the parasitic organisms. While this theory seems acceptable to explain *some* of the accompanying phenomena, there are discrepancies which appear to be potent evidence to contradict its validity. It is true that in *most* cases cutaneous anergy

is observed in conjunction with the demonstrable presence of large amounts of diseased tissue, containing of course, myriads of fungous cells. Also that the complement fixation test reveals a high concentration of the specific "antibody" which enters into that test. This is the *only* substance which has as yet been demonstrated by any test to be present consistently whenever the disease is in a severe, extensive phase. However, there is no evidence *for,* and *much evidence against the belief that complement fixing* antibody, in any quantity whatever, can neutralize skin-test reacting potentiality, either temporarily or permanently. Also, some cases of coccidioidomycosis exhibit cutaneous anergy continuously during many months or even years, while all evidence, clinical as well as serological, points to only a *small* residual focus of active disease. Conversely, many patients with coccidioidomycosis retain a high degree of cutaneous reactivity, even *hyperergy* over long periods of time during *which* extensive *amounts of* heavily diseased tissues are easily demonstrated.

It seems somewhat more logical to ascribe this anergy simply to the failure on the part of the patient to develop or to maintain reactivity, perhaps because of the same defect in his immunologic mechanisms which causes him to fail to develop immunologic *resistance.*

Having pointed out the value and the limitation of coccidioidin skin testing in *diagnosis,* let us now consider *another aspect of its usefulness, its value in prognosis.* It has long been recognized that "Valley Fever" is a *"once in a lifetime"* occurrence, belonging in this regard in the same class as measles, mumps, varicella and variola in which one well developed attack confers lifelong immunity to *subsequent reinfection. In contrast to these other* diseases however, by utilizing the *coccidioidin* test we can determine *which* individuals in the general population

have had *coccidioidomycosis* and are hence immune to further acquisition of *that* infection. We need *only* be cautious in ascertaining that those individuals who react positively to the test do not possess the disease in an active form at the time the test is made; if they do not, then we can predict that they will not *subsequently* become infected if exposed This is a definite *departure* from the behavior of the tuberculin test, for positive reactors to it *can* be reinfected with tuberculosis, and are, indeed, often affected more seriously by such reinfection than those who do *not* so react.

As has been pointed out, those patients who present erythema nodosum or multiforme as a part of their early coccidioidomycosis regularly react violently to coccidioidin, yielding positive reactions to as little as a 1-10,000 dilution. These persons are resistant in the *highest degree* to the *serious*, granulomatous form of the disease, and hence *never* succumb to it. They uniformly recover rapidly and are thereafter apparently highly immune to reinfection. No case reported contains *definite* evidence against this statement, although the following additional argument is necessary. The author has personally observed one case in which the history indicated a likely exposure to *Coccidioides* and the patient's principal presenting symptom was a large, tender, reddened swelling on the antero-lateral surface of a leg, entirely typical clinically of erythema nodosum During the first interview the author reassured the patient, stating that if his disease were shown to be coccidioidomycosis, as seemed likely, his prognosis was *very good*, citing his belief that in that disease erythema nodosum *always* indicated rapid, complete recovery and subsequent immunity. This dictum had to be tentatively reversed with considerable embarrassment 48 hours later when it became evident that the patient's

skin had *failed* to react to coccidioidin in a 1-1000 dilution and this reversal later confirmed when a 1-100 dilution had also failed to elicit a response. Liquid material aspirated from this lesion then revealed *numerous spherules* of *Coccidioides,* as did a histopathologic preparation. Thus, the lesion presented by this patient was *not* erythema nodosum, warranting a good prognosis, but the first of several extrapulmonary lesions of the disseminated form, indicating a poor outlook. *Amputation of the leg* was eventually resorted to before this patient became well. The author knows of two other similar cases in which patients presenting acceptable erythema nodosum by *clinical* opinion, eventually manifested the serious, granulomatous disseminated disease; in both instances there is reason to believe that this clinical opinion was *erroneous,* and that instead these nodular lesions were *already* of the *disseminated* type. It must also be pointed out that even though erythema *nodosum* or *multiforme is* actually present concomitantly with coccidioidomycosis, the *allergic* portion of the syndrome *may* have arisen from yet another infection, or more plausibly, from a drug administered for nonspecific reasons. It is necessary, therefore, to confirm the clinical impression that *true allergic* erythema nodosum or multiforme is present and is being *caused* by *Coccidioides* by showing the skin reactivity to coccidioidin *to be high,* before utilizing this feature as evidence to support a good prognosis. When in doubt, failure to reveal the organisms by smears, cultures and biopsy study of histopathologic preparations *of such* lesions will be helpful evidence of their allergic nature by *denying* the presence of the serious, granulomatous form.

From the foregoing it can be seen that there is a wide range of reactivity to the intracutaneous injection of coccidioidin in patients known to possess the disease, vary-

ing from an *intense* reaction to the material in 1-10,000 or to 1,000 dilution on the part of those exhibiting erythema nodosum or multiforme, and who *uniformly* recover and are therefore *immune* to reinfection, to *no response* whatever to the undiluted material on the part of those about to *die*. Also that it is among the group composed of those patients *between* these extremes, who develop only a poor reactivity during the acute primary pulmonary form of the infection or who exhibit it for a while only, that dissemination most often occurs, resulting in the serious form

It is the above facts which support the thesis that the *clinician* may rely upon the degree of reactivity to the cutaneous test, quantitated roughly by using several dilutions, as a measure of the *fighting power* which the patient is utilizing *against* the disease at the *time* the test is performed. As has been shown, this reactivity does not necessarily remain at the same level for any certain length of time, it may *increase* or *decrease*. Therefore, the test should be repeated at intervals of from 2 to 6 weeks. If the reactivity decreases or disappears in so short an interval, it should be interpreted as indicating a significant decrease in the patient's ability to resist the disease, and vice versa. It is not certain just how accurately one may rely upon this attempt at quantitation with regard to the result of a single test, but a *trend* established in one or the other direction over several weeks may be so used with great confidence. As a *clinician's yardstick*, the *extremities* of this procedure are clearly defined and easily seen and interpreted The *intermediate* markings are less distinct, even at times questionable. There is room for much study before they can be standardized and their true significance evaluated

Those scientists who have extensively studied human tuberculosis will find the foregoing concept difficult to accept as true, recognizing in it the counterpart of a thesis formerly held to be valid with relation to tuberculin testing, but later amply contradicted by several well established facts. The author's reasons for maintaining this concept in spite of such evidence will be presented later, when, after delineating further useful applications of testing procedures utilizing coccidioidin, the nature of this testing material will be more appropriately discussed in detail.

Chapter 4

COCCIDIOIDOMYCOSIS AS A GUIDE IN THE STUDY OF INFECTIOUS DISEASES—THE SIGNIFICANCE OF THE SEROLOGIC TESTS USING COCCIDIOIDIN (COMPLEMENT FIXATION AND PRECIPITIN)

OUT OF THE large number of persons who acquire the acute primary pulmonary form of coccidioidomycosis only a small percentage (about one per thousand) fail to develop a complete and lasting immunity, and these tend to proceed to the acquisition of the disseminated type so dangerous to life. It has been mentioned that among these individuals there is considerable variability in the reactivity to the intracutaneous test, some reacting well, some for a short time only and some minimally or not at all. At first glance, it appears that in this group there are some examples of exceptions to the rule that a positive response can be used as a measure of the patient's immunologic resistance to the disease. However, it has already been pointed out that the level of resistance is subject to change in either direction, especially in the early phases of the disease, and it must be concluded that in *these* persons the disease swiftly becomes sufficiently extensive to overwhelm an otherwise *initially* satisfactory degree of developing immunity.

The Complement Fixation Test Using Coccidioidin As the Antigen

Thus it is evident that an accurate prognosis cannot be obtained by merely measuring the fighting power which the patient is mobilizing *against the disease*, it is equally necessary to be able to measure the fighting power of his *adversary*, which is the severity and virulence of the infection and the extent to which it has progressed at any particular time. For this vital information clinicians have learned to rely upon the reaction to another test utilizing coccidioidin; in this examination, it serves as the "antigen" in the fixation of complement, participating in a reaction carried out in a fashion exactly similar to the quantitative Kolmer modification of the Wassermann test. Here again, as was found with the skin test, a wide range of reactivity is encountered, necessitating the use of serial dilutions of the patient's serum eight or ten times (1-128 to 1-512) if the end point is to be determined. Specimens reactive into the twelfth tube in such a series (1-4096) have been found.

That complement fixation could be demonstrated in coccidioidomycosis was first reported by Cooke in 1915 with the comment that the results were "inconsistent." Better success was obtained by Davis, but still the reaction was believed to be unreliable, probably because his antigen was heated in a manner now known to be deleterious. Later workers, notably groups under C. E. Smith and Kessel obtained materials capable of giving consistent results.

Experience covering tens of thousands of tests on thousands of patients* have consistently revealed a close

*Charles F Smith, M.D., and his collaborators alone had performed 21,000 such tests from 1940 to 1950 as well as an equal number of precipitin tests.

relationship between the degree of complement fixing reactivity (titer) of a patient's serum and the degree of severity of his disease at the time of the test. As with the analysis of the skin tests these facts are thoroughly established as valid at the *extremes* of the range, a large *percentage* of patients who have the acute primary pulmonary form in a mild degree showing *no* complement fixation titer at all, and none of them very much; while *all* of those dying of the disseminated form yield sera capable of fixing complement in *high dilutions*. Here also, as with the skin test, it is somewhat more difficult to discern the *intermediate* markings on this "measuring stick." Almost all of those patients who recover from the primary pulmonary infection without suffering dissemination do not ever exhibit serum reactivity in a dilution of more than 1-16, and this titer tends to fall to zero as recovery takes place. Almost all of those who *do* sustain dissemination yield sera capable of reacting in dilutions beyond 1-32, and this titer tends to increase as the dissemination becomes more widespread, and the extent of the disease increases.

It is necessary to discuss some discrepancies here. Persons having only one small focus of disseminated disease, for example a single small skin lesion, may show complement fixing reactivity in a low titer only, perhaps in dilutions of only 1 to 4 or 1 to 8. Coccidioidal meningitis deserves special mention, since such patients frequently die while still showing low complement fixations titers. Thus it is somewhat misleading to state that the "titer measures the *severity* of the disease": it is probably more correct to say that it is the "*volume* of the diseased tissue which is measured" (C. E. Smith). In meningitis, for example, a small *volume* of diseased tissue can kill rapidly, or cause such fibrotic changes as will *eventually* cause

death, while a lesion of similar small size in the *skin or* another less vital organ would be more likely to be successfully combatted and the patient to recover completely eventually. Patients of *each* of these two types might reveal the *same* low serologic titer to complement fixation with coccidioidin.

This author would like to suggest one further change in the wording of this concept, more because of certain observations in relation to the disease, North American blastomycosis, than to coccidiodomycosis, but consistent with the latter as well; this modificaion is to state it thus. "the complement fixation titer measures the number of organisms of the parasite actively engaged in producing the disease at the time of the test." For example, a patient who has extensive erythema nodosum–multiforme due to coccidioidomycosis may be *"severely"* ill, and he obviously has a large volume of *"diseased tissue"*; caused by coccidioidomycosis, but most of this, being allergic in nature contains no living organisms, and the complement fixation titer is accordingly usually low. There are also examples of chronic disseminated coccidioidomycosis of the skin of long duration in which the volume of involved tissue would be expected to yield a higher complement fixation titer than that actually found: an explanation of this discrepancy is furnished by the discovery by histopathologic examination of such tissue that *Coccidioides* organisms are present in but *very small numbers only*. Although rarer, this phase of the disease is entirely analagous to the chronic cutaneous form of North American blastomycosis (see page 123).

In this author's opinion it may even prove to be true that the material present in the serum which acts conjointly with coccidioidin to fix complement is actually not produced by the *patient*, but by the *Coccidioides or-*

ganisms as a part of their activities while they are actively engaged in producing the infection.* It has long been recognized that "antibody" produced by injecting an "antigen" into an animal's body is likely to be closely related in chemical structure to the antigen itself. In most such instances, however, it would indeed be naive to believe that the antibody is *derived* from the antigen itself, rather than *stimulated* by it since it is known that the *quantity* of the former which is produced is tremendously out of the range of the quantity of the latter which is injected, and *continues* to be made long after the stimulating material has disappeared. However *neither* of these facts is applicable to coccidioidomycosis, where there is *never* more "antibody" capable of fixing complement than that which can logically be considered proportional to the number of living organisms present and in the *act* of producing the disease. Perhaps they simultaneously produce the complement fixing substance as well, and its titer declines when they become less active, disappearing entirely when the disease is cured. After such a "cure" the human body apparently has no "memory" of how to make this complement fixing antibody, thus the "anamnestic reaction" so typical of immunologic and allergic processes in general seems peculiarly absent, a circumstance which must be considered highly significant.

Let us now examine the manner in which this quantitated complement fixation reactivity may be utilized by the clinician. Within certain small limitations which will now be discussed, it is of definite diagnostic value; that is to say, when positive, it indicates the *presence* of active disease at the time of the test. It is highly specific, showing no tendency toward yielding false positives in

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*Or such a product may be needed for combination with a serum component molecule for molecule

other diseases except some of the other deep mycoses, such as histoplasmosis, and perhaps blastomycosis and sporotrichosis. Even these cross reactions are not very well established, and better standardization of materials and procedures may add to the degree of specificity; the utilization of other diagnostic methods such as cultures should clarify other instances. It will also usually be found that these *cross* reactions will occur only in the less diluted fractions, while the *true* reaction of the *disease* which is *actually present* will be positive in a significantly *higher titer.*

Two other possible sources of error must be explained. The first is due to the fact that the ability to react to the complement fixation test is not acquired *immediately* after the onset of the disease; in fact it is delayed in is appearance more than the skin test reactivity, sometimes even for as long as 3 months. Hence the complement fixation titer cannot be relied upon for *diagnosis* nor *prognosis* in the *early phases* of the infection since it may at that time yield a "false negative." Fortunately this gap is filled to a large extent by yet another coccidioidin test, the "precipitin" test, to be discussed soon. The second discrepancy occurs because there is reason to believe that complement fixation reactivity may persist in a low or moderate degree, (perhaps, as much as to a 1-8 dilution,) for years after the disease has actually become cured. While this appears likely to be true it is *dangerous* to be too willing to *accept* this an an explanation in any individual case and therefore to allow the patient to become physically active and to lapse from observational control, since many such instances are known *in which the infection has eventually proved to have been* only *quiescent*, later becoming severe again and proceed

ing to fatality. This decision is especially fraught with danger if it is made in the face of the knowledge that the patients skin test reactivity is low or absent, indicating a poor state of immunologic resistance.

Within these limitations a positive reaction to the complement fixation test means the presence of coccidioidomycosis in an active phase at the time of the test.

In prognosis also, this test is of great value, since it serves the clinician as a reasonably accurate measure of the number of active coccidioidal organisms which the patient must combat. Within the limits previously outlined, at intervals of a few weeks, the *interplay* between the results of this test and the intracutaneous test can furnish the clinician a more reliable guide to prognosis than *any other combination of clinical opinion, physical signs, x-rays, or laboratory studies* As an example of this usefulness, it sometimes happens in the primary pulmonary type of the infection that a patient appears to be extremely ill, presenting a clinical picture by x-ray, laboratory and physical examination which would surely indicate a fatal outcome if the disease were any other than coccidioidomycosis. However, in the absence of signs of central nervous system involvement, a hopeful attitude is to be maintained as long as the *skin* test reactivity remains *high* and the *complement fixation titer low* or absent Conversely, a patient known to have had the disseminated form may appear temporarily to be completely recovered by all other means of obtaining a prognosis, but if the *skin* test reactivity is *low* or absent and the *complement fixation* remains *high* or is observed to be increasing on succeeding tests, *further danger is to be anticipated* Under these circumstances death has been accurately predicted in several instances observed by this author in spite of much clinical evidence to the contrary

3 months. However 50% of these persons will show *precipitin* reactivity by the end of the first week of illness and 90% by the end of the third week. If the physician is fortunate enough to observe the *conversion* from *negative* on first testing to *positive* a week or two later, it will be helpful in determining the time sequence and may be accurately taken to mean that a coccidioidomycotic infection is present and in a *very early stage*. It must also be recalled that in a large percentage of *mild* cases, no complement fixation reactivity *ever* develops, nevertheless most of these *will* show precipitin reactivity. This is especially valuable in those cases of acute pulmonary infection of undetermined origin in which coccidioidomycosis is *suspected* but in which the reaction to the intradermal test is positive at its *first* application. The problem then presents itself because intradermal reactivity usually endures for many years, hence it cannot be stated that such patients who react positively have coccidioidomycosis in an *active* form *at that time;* the reactivity may not have resulted from the *presenting* pulmonary illness but from a *previous* infection, leaving the present diagnosis *still* in doubt. On the contrary, precipitin reactivity does *not* so persist, it begins to decline as early as the third week and has *never* been demonstrated after the seventh month, regardless of the status of the disease or the fate of the patient Hence when a positive precipitin reaction is obtained it means that the disease is *active at that time.* It is obvious however, that a *negative* reaction does not *rule out* the presence of the disease, since it may yet be too early for reactivity to have developed, or conversely, too *late* because such reactivity disappears after a few months even though the disease continues.

The reactivity to the precipitin test apparently has no value in prognosis; it is not consistently higher in

serious cases than in mild ones, and disappears after a short period of time regardless of whether the patient is destined to die or to recover. It has not been subject to quantitation, either by using serial dilutions of serum or of antigen although the latter of these two modifications is employed for increased clarity of the readings.

Chapter 5

COCCIDIOIDOMYCOSIS AS A GUIDE IN THE STUDY OF INFECTIOUS DISEASES—THE CHARACTERISTICS OF COCCIDIOIDIN, AN ALMOST IDEAL TESTING MATERIAL

THERE are many examples of diagnostic testing procedures in which the materials utilized are of such doubtful composition that it is surprising that information of any value whatever can be obtained. For example "lepromin" for the Mitsuda reaction contains all of the soluble components to be found in human lepromatous nodules. It is difficult to imagine a more heterogenous mixture than this, containing as it does extractives from all tissues, healthy as well as diseased or killed, which are contained in epidermis, cutis and subcutis, and in addition components derived from blood, pus, enzymes and bacilli in all stages of degradation Yet this material has been shown to be reliable in serving to distinguish between the lepromatous and tuberculoid forms of leprosy.

Purer than this by far is "old tuberculin," cleanly produced from pure culture on simple medium; yet this substance is still a complex mixture separable into several components. A still purer form, known as the "purified protein derivative" serves well in carrying out the only really significant allergic testing procedure in con-

nection with tuberculosis, the intracutaneous "tuberculin" test.

One of the principal reasons why coccidioidomycosis has so much to offer to students and investigators in the field of immunology is the fact that it is easy to prepare from pure cultures of the fungus a testing extract remarkably pure, homogenous, and stable, and capable of acting as an antigen in at least three highly informative reactions, maintaining in all of them a high degree of specificity for its corresponding disease. It cannot be claimed that coccidioidin is more fully understood solely because there has been more effort spent in its study; in a large measure it is because it is inherently a relatively uncomplicated substance comparatively free of undesirable characteristics no matter how it is made. The disease coccidioidomycosis is apparently equally fortuitously endowed with more easily understood phenomena than other diseases.

Coccidioidin represents all of the antigenic material that the fungus *Coccidioides* immitis can produce when growing in a pure culture, on an artificial medium which *in itself* cannot contribute any antigenic substances. The high degree of specificity in all three coccidioidin tests is due in a large measure undoubtedly to the ease with which we can prepare such a simple and pure extract of the causative organism of the disease. In this regard the following points are important.

There is almost no evidence to indicate the presence of any variability in antigenic productive power among different "strains" of *Coccidioides;* even this small doubt is usually cancelled out in practice by using a number of such "strains" concomitantly. The nutritional requirements of the fungus are so simple that it will grow in a solution containing only sodium acetate and ammonium

chloride, and form coccidioidin of good quality, although the quantitative yield is impractically low for general use. The medium *usually* employed for its production contains in adidtion to inorganic salts, only citrate ions, glucose, glycerin, water and a single organic component which may be considered even remotely related to protein, the amino-acid amide, asparagine, a simple compound, $COOH \cdot CHNH_2 \cdot CH_2 \cdot CONH_2$, in the levo-rotatory form. Except for some variations in concentration this medium is identical with that used in the production of "old tuberculin." All of these substances can later be separated by dialysis from the coccidioidin[*] produced ithout changing its activity, but it was found by experi-ntation that this is not actually necessary in practice ce they are not in themselves antigenic nor capable of yielding "false positive" reactions

There is considerable evidence to show that coccidioidin is a single substance, a large organic molecule, principally of polysaccharide structure,[†] but containing a small percentage of nitrogen, (3.2%, 0.6% of which is in the amino acid form) probably exhibiting several kinds of haptene groups. No protein was shown to be present by the routine Millon, xanthoproteic, trichloracetic acid, glyoxylic acid or buiret tests; but preliminary electrophoretic studies indicated that it might be present in a very small percentage (of the magnitude of 0.09 mg. per cc. (Seibert) but probably intimately connected with the polysaccharide molecule, perhaps constituting one of the haptene groupings). As will be shown later, it may be

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[*]The coccidioidin molecule is too large to pass through a collodion membrane

[†]Hirsch and D'Andrea, Hassid, Baker and McCready

intracutaneous testing in *other* diseases where specificity is not so easily attained.

Conversely, since the removal of nitrogen from coccidiodin removes its complement fixing ability, the application of heat or chemicals for purposes of sterilization should be *avoided* in the preparation of antigens intended to serve in *complement fixation* tests in other diseases. An antigen intended only for *this reaction* need not even be sterile (at least as far as the pathogenic fungus from which it is derived is concerned), except for the prevention of deterioration in storage, a result which might be accomplished by other means such as deep freezing or *lyophilization. For complement fixing* antigens, perhaps the original culture medium might even be advantageously *enriched* with nitrogen-containing subtsances in an attempt to make the resulting material *more protein* like in structure.

Another advantage possessed by coccidioidin over other similar testing materials is its great degree of stability. Although it is true that the complement fixing component can be destroyed by heat, the temperature must be carried far higher than that which will ever be encountered in ordinary use, that is unless heat is purposely applied. (The earliest workers sterilized their coccidioidin by autoclaving, hence they were unable to obtain satisfactory complement fixation consistently.) Even this heat-labile component retains its potency for years without refrigeration Skin test and precipitin reactivity were observed to be retained (without any decrease demonstrable by careful standardization) in a specimen of coccidioidin carried in the glove compartment of an automobile in a desert climate for 6 years and subsequently kept for 3 years at room temperature (C. E. Smith) Even the various *dilutions* remain stable at room temperatures

for months and, probably for years. The only factor yet observed to be able to cause loss of potency is contamination of the salution with bacteria. Not only does this destroy the coccidioidin, but the resulting bacterial products can cause false positive reactions in the subsequent tests. The slightest degree of turbidity of the material therefore renders it unreliable because it usually indicates contamination.

Coccidioidin also has the quality (advantageous in one regard, disadvantageous in another) of being unable to induce sensitization in the normal animal body. It is thus an incomplete "antigen." Animals have been given large quantities by the subcutaneous and intraperitoneal routes without gaining the slightest reactivity to any of the three tests. Certain persons have also received what would seem to be adequate amounts subcutaneously with similar negative results. The *intravenous* route has been used only by some of those advocating coccidioidin in the *treatment* of the *active disseminated disease* (Jacobson) and the amounts have been exceedingly small; it is thus not certain whether reactivity could be produced by this route in *normal* individuals It must be admitted that immunization can be much more efficiently induced by intravenous administration of antigens than by the subcutaneous route in certain *other* diseases (pneumococcus infections for example) * Admission of this possibility does not infer that it is also likely that intravenous administration of antigens to animals *already infected* with the corresponding disease could duplicate such success. Further comment on this phase of the study will be made

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*However, Stewart and Meyer believe the intracutaneous route is preferable to intravenous inoculation, although it is undeniably true that the effective immunity acquired so easily and almost universally by normal individuals does not depend on this mechanism

later in the consideration of vaccino-therapy in the paragraphs on treatment (see page 60).

However, the activity of the fungus within the *infected* body *does* produce an effective skin test hypersensitivity and serologic ability to react to the complement fixation and precipitin tests, (in all but a very small percentage of instances). Hence, under these actively *parasitic* circumstances, the fungus has antigenic abilities beyond those possessed by the only *extract* which has been yet recovered from its culture phase (coccidioidin).*

The *advantage* of *this incomplete antigenicity* of coccidioidin lies in the fact that it need not be withheld for fear of harming the patient or of sensitizing him so that future tests (either cutaneous or complement-fixing) will give false information. The material is safe except for the production of unduly severe local reactions when unduly high concentrations are injected intracutaneously in the early stages of the primary pulmonary type, and its ability under similar circumstances to precipitate the erythema nodosum—erythema multiforme type of "ids" (coccidioidids).

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*The staining procedures employed by Tarbet and Breslau on tissue sections indicate the presence of a phospho-lipid in the wall of mature spherules; this substance was not removable by fat solvents. It has also been observed that mature spherules when seen in histopathologic preparations are surrounded by a chronic granulomatous tuberculoid tissue reaction similar to that seen regularly in tuberculosis and reproducible by lipoid fractions derived from tubercle bacilli. This suggests that *coccidioides* may produce lipoid substances in this stage of its parasitic phase which it does not do in the *culture* phase. Tarbet and Breslau also demonstrated a mucopolysaccharide in the wall of small spores, but could find no cellulose. These protein-carbohydrate or lipoidal substances may be more closely representative of the total antigenic powers of the parasitic phase than the "protein free" polysaccharide called coccidioidin made from the artificial culture phase.

Chapter 6

COCCIDIOIDOMYCOSIS AS A GUIDE IN THE STUDY OF INFECTIOUS DISEASES–IMMUNOLOGIC ASPECTS

For those who still find it difficult to accept the claim that coccidioidin testing is a valuable diagnostic and prognostic procedure in coccidioidomycosis, the following additional argument is presented

The Intracutaneous Reaction

Let us first examine more fully the subject in general of hypersensitivity as evidenced by the reaction to the intracutaneous injection of specific materials. Identical responses both of the type called the "immediate flare" reaction and of the "delayed tuberculin" type have been observed to occur following repeated contact of bodily tissues with many different kinds of noxious materials such as those produced by or extracted from bacteria, fungi, viruses, rickettsiae, animal parasites, protozoa, foods and danders It seems unlikely that nature would furnish such a mode of reaction to animals so universally if it had no beneficial effect, and yet in most instances hypersensitivity appears to be harmful, at least it usually causes the animal symptomatic distress beneath which it is difficult to discern any benefit.

The most thorough investigation of the "delayed" type of hypersensitivity has been as it occurs in response to the tuberculin test. Such hypersensitivity was long regarded as an essential factor in acquired immunity to tuberculosis but in more recent years, convincing evidence has been assembled that there is no relation between the two. In support of this later view it is pointed out that in tuberculosis the degree of such hypersensitivity does not parallel the degree of acquired immunity; that the inflammation resulting from hypersensitivity does not prevent the spread of the bacilli which the inflammation of immunized tissue achieves; that effective resistance can be established without simultaneously establishing hypersensitivity; that acquired immunity can be passively transferred while hypersensitivity cannot; and finally, that acquired resistance persists after hypersensitivity has waned or has been abolished by desensitization (Rich).

Similar evidence has been assembled in a number of other diseases; so much in fact that it seems like heresy to maintain that in coccidioidomycosis the degree of immunity is clincally measurable by the hypersensitivity reaction. Many of the above mentioned factors however, have not yet been approached experimentally in coccidioidomycosis; the only one which seems to be reasonably established is that acquired immunity does persist after the reaction to the intracutaneous test becomes negative through years of slow decline.* There has been no evi-

*C. E. Smith has personally observed his own intracutaneous reactivity slowly declining during many years until he reacts minimally to no higher dilutions than 1:10. He has not acquired coccidioidomycosis again in spite of conclusive evidence that he has been adequately exposed since his reactivity became so lowered. Also among thousands of persons shown to be coccidioidin skin test positive years ago, no case of reinfection has been recorded, although many must have lost a large degree of such reactivity through the years.

dence to show that this decline in reactivity occurs because of the development of yet another antibody antagonistic to the one originally responsible for it (the so-called "positive anergy" postulated to explain the identical phenomenon in tuberculin testing). It is well to recall here that coccidioidomycosis, no "sarcoidal stage" has as yet been covered analagous to that thought by some authors to be a "stage" in tuberculosis accompanied by "positive anergy." Sarcoid has, however, been observed occasionally in *conjunction* with coccidioidomycosis, and apparently not directly related to it. Before accepting this *one* situation in which skin test reactivity fails to parallel ability to resist the disease, as proof that the two are in *no* way related, it is necessary to consider as another possibility, that such hypersensitivity may be but a *stage* in the development of immunity, later becoming gradually converted into a process non-reactive in this regard. This theory gained considerable acceptance in this regard with tuberculin testing and other similar reactions, before being discredited, later. As will soon be shown, this conclusion does not necessarily render the theory equally inacceptable in coccidioidin reactions.

There is, however, one factor of tremendous potential importance in which coccidioidomycosis and its intracutaneous test differs from most other conditions in which a similar reaction takes place. The delayed tuberculin type of reaction occurs in these other instances as a response to the intracutaneous injection of various specific substances, all of them *proteins*. In coccidioidomycosis, by contrast, it is a pure *polysaccharide*, *coccidioidin*, which excites the same type of response. There are some other exceptions, notably some other fungus extractives which are also polysaccharides and which be-

have similarly (e.g., trichophytins and some blastomycins, sporotrichins and nocardins).

Referring back to the paragraphs on the nature of coccidioidin it will be recalled that there is still a possibility that coccidioidin is not entirely free of protein structures, although the amount must be small. It must also be pointed out however that *autoclaving coccidioidin* removes most if not all of the nitrogenous content from the molecule *without lowering its reactivity for the skin test.* Protein cannot be present without nitrogen, and *hence the skin test almost certainly depends on a protein-free component.*

Conversely, tuberculin produces its cutaneous response by virtue of its *protein* content; indeed, the most highly purified tuberculin is called the "purified protein derivative" (P.P.D.). Thus the fact that it is established that the delayed tuberculin type of cutaneous hypersensitivity has no relation in degree with immunity *in tuberculosis* cannot logically be used as argument against a similar possibility in coccidioidomycosis. (To make such a comparison is like the old humorous question "Is it warmer in the country than it is in the summer?") There must be at least a reasonable degree of similarity between two phenomena before they can be constructively compared and conclusions drawn. It is important, then to remember that it is pure tuberculo-protein that is the measuring stick in the *tuberculin* test; in the *coccidioidin* skin test it is a pure protein free-polysaccharide.

Also of interest is that the "acute splenic tumor" *which occurs in many bacterial infections is not observed* in coccidioidomycosis. In this reaction the spleen is filled with large mononuclear cells with large vesicular nuclei and basophilic cytoplasm which some observers consider to be phagocytes and others lymphocytes. The cause of

this phenomenon is apparently always a protein substance; some proteins can even produce it unaccompanied by infection. It would seem from these facts that *Coccidioides* may not produce much if any specific protein while growing as a parasite in the animal body just as it probably does not do so when grown in artificial culture. There is some conflicting evidence here, in the work of Tarbet and Breslau (see footnote on page 48)

It must be also pointed out that there are other important examples of the different potentialities of proteins and polysaccharides. Thus, some specific polysaccharides possess immunizing power in other diseases in which the corresponding proteins do not. For example, pneumococcal polysaccharide will serve to immunize an animal without concomitant development of hypersensitivity; pneumococcal protein produces only hypersensitivity.

It is noteworthy that coccidioidin is usually produced by culturing the organisms in the same asparagine synthetic medium that is used for making old tuberculin. Simple filtration yields only protein from cultures of *tubercle bacilli* grown in this manner whereas similar treatment of *Coccidioides* cultures produces only a low-nitrogen-containing polysaccharide. It is true also that additional antigenic substances such as polysaccharides and lipids may be obtained from the *bodies* of the *tubercle bacilli* themselves, by crushing or grinding them with the culture medium before filtration. But when *Coccidioides* cultures are treated in the same way, no new antigens are added to the filtrate *

*Jacobson claimed that his method of manufacturing coccidioidin (by grinding in a ball mill the mycelial mat of the cultures with the filtrate) yielded an additional component which he called an "endotoxin" The greater part of the evidence points away from this belief however Smith and his associates tried unsuccessfully to find any differences whatever in coccidioidin produced by all conceivable methods from artificial cultures of *Coccidioides*

The tuberculin test as it is used today by employing tuberculo-protein has no value aside from its ability to designate certain persons as having had a tubercular infection either past or present. At this point its potentialities apparently have been exhausted, but it still continues to be investigated and used more than seems indicated. It is possible that the substitution of one of the *polysaccharides* derived from tubercle bacilli as mentioned above might yield a testing procedure revealing a cutaneous hypersensitivity which parallels immunity, which could then serve as a valuable prognostic aid, even as coccidioidin serves in coccidioidomycosis.

The Complement Fixation Reaction

The great weight of experience with the complement fixation reaction, particularly as it applies to syphilis, makes it also seem heretical to claim that in coccidioidomycosis the degree of reactivity is directly related to the severity of the disease and that by repeated tests its clinical course can be charted That this view may still prove to be valid with coccidioidomycosis must be admitted, however, when the antigens are compared—or, more appropriately, contrasted In the test as applied to *syphilis* the antigen is not even remotely related to the *disease* nor to the *organism* which causes it; in *coccidioidomycosis* it is a *pure extract* produced by the causative *organism*. Furthermore the antigen in syphilis is a *lipid* and it ought not be assumed as inevitable that there should be similarities between the reactions evoked by it and the reactions caused by the *polysaccharide* antigen of *Coccidioides* whether or not it is entirely free of *protein*. It is certainly possible that if a pure culture of *Treponema pallidum* could be grown on such a simple medium as that used for the production of coccidioidin, a substance *might* be

yielded which could duplicate the prognostic results when applied to syphilis.*

The Precipitin Reaction

The precipitin reaction does not seem to be concerned with prognosis or immunity in any manner, and need not be treated further here.

The Nature of Immunity in Coccidioidomycosis

In the majority of infections in which the mechanism is understood, it is evident that acquired resistance is the result of the formation of antibodies by the tissues as a result of contact with the organisms. These antibodies are highly specific globulins (proteins) apparently closely related chemically to the antigens which stimulated their production The abilities of these antibodies to attach themselves to the organisms and cause them to become mutually adherent, to interfere with their respiration or metabolism, or to cause them to be more easily attached to and engulfed by phagocytes, are integral parts of immunologic processes. Apparently, sometimes a *single antibody* can cause several or all of the reactions known as complement fixation, precipitation, agglutination, lysis and opsonization under appropriate circumstances. In only a

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*Since the original writing of this chapter, Portnoy and Magnuson have prepared an antigen from *T pallidum* organisms obtained from rabbit testes and found it capable of yielding a complement fixation reaction with syphilitic human sera This reaction was shown to reveal the presence of an antibody differing from that involved in the Wassermann test ("reagin") since it could be obtained with syphilitic sera after this "reagin" had been removed. Perhaps this new antibody, revealed by this new "protein" antigen in the complement fixation reaction may prove to parallel the "amount" of syphilis present at the time the test is made It will be interesting also to learn whether polysaccharide fractions can be obtained which could serve as a "skin test" in syphilitic persons

few diseases can the "antibodies" actually *destroy* the organisms without the assistance of phagocytes.

A question yet to be answered is whether or not the immunity so quickly and easily acquired by most persons exposed to coccidioidomycosis is the result of the production of specific antibodies effective against the disease. In the study of this disorder it is a handicap to be unable to produce the fungus in pure culture entirely in the spherule stage in which it exists while a parasite in the body. Experiments in vitro with such material, if it were available, might uncover antibodies not yet recognized such as those capable of producing lysis, agglutination, increased susceptibility to phagocytosis or even destruction of the spherule stage of organisms directly. Spherules have indeed been *produced* in culture, but not separable from the mycelial form in the usual artificial cultures or from the complicated proteins necessary in the medium to support the spherule stage in tissue culture or in embryonated eggs.

By the methods thus far developed no effective antibodies have as yet been discovered in the circulating blood of persons who have demonstrated resistance against the disease, but this does not deny the possibility. In fact, there are suggestive phenomena. For example, multiple transfusions of whole blood have been employed with some apparent benefit in disseminated coccidioidomycosis. It is very likely that in many instances such blood was obtained from donors who have become immune *to reinfection* by recovering completely from the primary pulmonary form of the disease; actually, this is almost certain to have happened in utilizing blood from banks drawing supplies from the populace in endemic areas. *It is* entirely plausible that such blood may have helped the patient by furnishing specific antibodies A concerted

attempt has not as yet been made, however, to ascertain whether or not larger amounts of blood obtained from donors who are *selected* because of high *skin test reactivity* to coccidioidin will cure cases of coccidioidomycosis or consistently improve the prognosis. If a pilot study utilizing such selected whole blood should prove promising, attempts could thus be made to transfer a passive immunity by utilizing gamma globulin fractions or other portions separated from the blood of immune persons by the several methods which have been perfected during recent years. Electrophoresis, the ultracentrifuge, Seitz filtration, lyophilization and chromatography should assist in the identification, purification and storage of any such antibodies as might be found to exist. If, by injection of an appropriate antibody, a *passive* immunity could be conferred on those few persons who do not develop *active* immunity spontaneously, perhaps they could be kept alive long enough to do so. Only immune serum from *human* donors could be used for this purpose. In *acute* diseases, serum derived from *animals* can be utilized, since the patients can be helped materially within a few days. In chronic diseases, however, antiserum from other animal species is probably useless, since the heterologous proteins have time to induce antibodies against themselves which destroy or precipitate them within a short time after they are given in subsequent injections and before the patient can be materially assisted. Since antibodies are *proteins*, even if they could be highly purified they would still carry such species-specific antigenic power of self-destruction. However, specific immune sera from animals might be helpful in another way (see page 64, last paragraph).

It must be concluded that there are *some* antibodies present in the *Coccidioides* infected body, for how else

can be explained the high degree of specificity of the intracutaneous test and the complement fixation and precipitation reactions? Also these antibodies must *differ* from *each other* since *each* is present in individually variable quantities and at *different times* during the course of the disease, overlapping much too much to be considered capable of transition, one to another. The antibody responsible for the *complement* fixation reaction, for example, cannot be useful in supplying acquired immunity, for its presence in increasing amount indicates a *serious trend in the disease, also, immunity persist long* after *that* antigen disappears. The antibody involved in the precipitation test is present for only a few months whether the patient is destined to die or to recover. *That* antibody, therefore, can have nothing to do with immunity. *Both of these antibodies are present in serum* and can be transferred passively, even through the placenta.

There may be yet other antibodies present in serum which must be considered. Frequently, when coccidioidin is introduced intracutaneously, there is an immediate flare *reaction with the development of a wheal.** This is the same type of response as that usually interpreted as being highly specific and significant of so called "atopic" hypersensitivity when it is encountered in the course of testing the skin with extracts of foods, danders, pollens and the like. *The antibodies involved* in this process are usually spoken of as "circulating" antibodies. *Unfortunately,* although this phenomenon is often observed in coccidioidin testing, it has not been possible to correlate it with the acquisition of immunity, or indeed, in any other specific manner with the disease itself or with its progress. Thus even if its specificity for coccidioidomycosis could be

*First reported by Hirsch and Benson in 1927

proved, this antibody is not likely to be one which contributes resistance to the disease.

While some or all these several substances are undoubtedly present in the serum of the infected patient and are correctly termed antibodies, each being a *body* active *against* a specific substance; they are not *antibodies* actively effective *against* the *disease*, and hence they are not capable of being of benefit to the patient by helping him to resist it. However, there is still a possibility that truly effective antibodies may be present in appropriate sera.

The presence of "neutralizing" antibodies has been conclusively demonstrated in the sera of persons after contact with various pathogenic organisms, particularly with viruses. When mixed with adequate amounts of virulent organisms prior to their injection into susceptible animals these antibodies are capable of preventing the transmission of the disease. Studies of this phenomenon could be made by utilizing artificial cultures of *Coccidioides* containing infectious arthrospores, as well as virulent organisms in the spherule stage derived from infected animal tissues, titrated against sera from patients known to be able to resist coccidioidomycosis

It should also be interesting to explore the possibility of the presence of a circulating antibody by utilizing the immune adherence phenomenon recently demonstrated in several diseases by Nelson This reaction apparently occurs over a wide spectrum of microbial infections, having been observed with *T. pallidum, D. pneumoniae, Shigella paradysenteriae, Salmonella typhi, Micrococcus aureus* and *Mycobacterium tuberculosis* While fungus diseases have not as yet been studied, it seems likely that they might behave similarly

It is also worth pointing out that even though the spherule stage of Coccidioides is *not* culturable on simple

media and separable from its components, concentrations of such spherules which *could* be obtained from heavily infected animal tissues would serve in many investigations in which a high degree of purity is not *necessary*. The phenomena of lysis, agglutination, opsonization phagocytosis and immune adherence to erythrocytes could probably be searched for adequately by this means, and antibodies might thereby be revealed.

The only evidence as yet obtained indicating that in coccidioidomycosis there is an antibody intimately connected with immunologic resistance to the disease, is that the ability to respond to the intracutaneous coccidioidin test closely parallels the possession of such resistance. There is certainly as yet no evidence to show that this antibody *is* the means by which immunity is developed, but it is equally true that it has not been sufficiently investigated to allow it to be excluded. This antibody cannot be passively transferred nor does it pass the placenta; hence it is concluded that it is not present in *serum*. It must be *fixed* to some type of *cell*. If *this* antibody *is* the one actually responsible for furnishing effective acquired immunity, it is strange that its presence is manifest principally in the *skin* where it is least needed, and where it can function only in the poorest manner in resisting a disease almost always acquired by inhalation of the organisms. If assisting phagocytosis is its most beneficial duty, then why, it must be wondered, does it not concentrate near the littoral cells of the spleen, lymph nodes and marrow and the Kupfer cells of the liver, instead of close to the stretched-out endothelial cells of the skin capillaries which do not function as a part of the reticuloendothelial system, at least not immunologically

Perhaps this antibody is actually fixed to the cells of other tissues as well as to those of the skin. Intravenous

injection of coccidioidin into certain patients has caused a febrile response, classed as a "systemic reaction," which may be the equivalent of the cutaneous reactivity revealed by its intracutaneous injection; however, there is no delay of 24 to 48 hours as is characteristic of the latter. It is even possible that the cellular components of the *circulating blood* have this antibody fixed to them; whole blood transfusions might then furnish passive immunity while serum could not. Leucocytes have been particularly suspected of having this ability to carry fixed antibodies. It might be that the element of value in conferring passive immunity could be transferred by injections of "buffy coat" at present wasted entirely in preparing lyophilized serum.

It must be mentioned that in many antigen-antibody mechanisms it has been established that the ability to react to the intracutaneous injection of a testing material by the production of an immediate flare or wheal is closely allied with the ability to produce the delayed tuberculin type of response, both reactions being considered to be but different stages in the development of the same process, the first representing the activity of the antibody while circulating, the other after it becomes fixed to cells. There is no evidence as yet to support this thesis as applicable to coccidioidomycosis (see also pages 222 and 223).

Up to this point we have considered the possibility of transferring a passive immunity against coccidioidomycosis. What about inducing an active immunity? So far, there have been no spectacularly successful methods discovered whereby an *active immunization* can be developed *artificially* in any animal to any disease which is *already actively present* in the body. All such methods act almost exclusively by conferring upon *normal* animals the power to resist subsequently an *original* acquisition of the infection.

Vogel, Fetter, Conant and Lowe have recently (1954) reported that they have succeeded in inducing a significant degree of specific immunologic resistance to coccidioidomycosis in guinea pigs by the injection intramuscularly five times at weekly intervals of 0.5 cc. of an antigen consitsing of *Coccidioides* spherules obtained from infected embryonated hen's eggs. Although comment at this early date on such a report cannot fail to prove to be premature, the following observations may be pertinent.

First: All animals (including controls as well as those receiving the immunizing injections) were alive 6 weeks after the challenging dose of *Coccidioides* arthrospores was administered by aerosol into the lungs. The *use of a dose sufficient to have produced at least a reasonable percentage of fatalities in the control group* would have been more impressive.

Second: All animals became infected and showed evidence of this at autopsy; although, in the opinion of the authors "the degree of macroscopic disease was less in the treated group." Thus the degree of immunity which developed was not successful in protecting any animal completely against acquiring the infection. There were, however, two instances of dissemination in the untreated group and none in the immunized group. This might be highly important in human infection, if we could be convinced that all human beings who sustain pulmonary *inoculation are as normal to begin with* as were these animals. It will be recalled that some workers believe that coccidioidomycosis disseminates only in those very few individuals who possess some inherent defect in their immunologic systems even before the inoculation occurs, and who might therefore presumably fail to develop immunity equally persistently from such an artificial means.

Third: It is highly important that these workers did observe that a very large proportion of the animals receiving the immunizing injections developed within 4 weeks the power to react to the intracutaneous test using coccidioidin, and that dissemination did not occur in any of these. This is in line with the thinking of those who believe that skin test reactivity parallels immunity to a useful degree for prognosis.

Fourth: The total quantity of the spherule antigen used as a vaccine for each pound of animal was very large, 2.5 cc. per 2 pound pig. For duplication in adult human beings this would require from 100 to 150 cc. per person, a quantity very difficult to produce for any large scale vaccination program from embryonated eggs.

Fifth: Any and all of the observations of the original workers reporting this phenomenon as well as those of this author may well fail to apply to human beings, in whom the mechanisms may not be at all similar.

Since coccidioidomycosis can be acquired only in small geographic areas, and yet can occur from the inhalation of a single breath of dust by a visitor from distant parts, epidemiologic control by mass vaccination of all who *might possibly* be exposed is entirely too impractical for serious consideration, especially when the *disease itself* so quickly and painlessly confers complete immunity upon all but one in a thousand infected persons. Thus, even if a method *could* be discovered whereby normal individuals could be furnished with active immunity, it would have but a limited application for those who are *known* to be destined to be exposed subsequently. This is probably just as well, since at present such a method does not seem likely soon to be developed.

Except for those diseases in which *toxins* play a major role in injuring the patient, and *antitoxins* delay death

until active immunity develops, immunologic victories over chronic diseases are rare. Most of them depend upon adventitious cross-immunity between diseases or upon discovering or producing a special strain of the organism that has diminished power to produce injurious infection yet retains its immunizing potency. There has been absolutely no evidence to show any variation in virulence in various strains of Coccidioides.* Even the transfer through several different species of animals in the manner which brought yellow fever under control does not noticeably attenuate the organism.

As has been stated, coccidioidin is not a complete antigen; and its injection into the animal body cannot induce the production of any demonstrable antibodies. When it is recalled that coccidioidin almost certainly represents the total ability of the *culture phase* of the organism to produce antigenic substances it is apparent that attempts to use material derived from such cultures as though it were a vaccine must fail. It is of course always possible that it might be combined with some other molecule so that it became potently antigenic while still retaining its specificity. It is also possible that a method can be discovered to yield a pure culture of the pathogenic tissue or spherule stage of the organism, an extract of which *could* prove to be a complete antigen or "vaccine." The impact of the statements in this paragraph will be most effectively appreciated when treatment is discussed in the succeeding Chapter.

It is at this point that "specific antibodies" derived from infected animals must be mentioned, since it is remotely possible that such materials could be combined

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*A few strains have shown a significant reduction in virulence as far as animal inoculations are concerned, but the human infections from which they were originally isolated were not any less severe than usual

with virulent *Coccidioides* organisms in sufficient quantities to prevent the acquisition of the disease when the combination is injected into normal persons as a vaccine which might still serve to immunize the individuals.

Chapter 7

COCCIDIOIDOMYCOSIS AS A GUIDE IN THE STUDY OF INFECTIOUS DISEASES—APPROACHES TO THERAPY

Vaccinotherapy

IS "VACCINOTHERAPY" OF BENEFIT? In the last chapter evidence was presented to show that methods by which *normal* persons could be artificially immunized against coccidioidomycosis must be considered *possible*, but would probably be impractical in most instances even if they could be developed efficiently. Some of these same facts appear to this author to indicate that the so-called "vaccinotherapy," advocated for the treatment of disseminated coccidioidomycosis by Jacobsen and by Stewart, is probably of no value to the patient and may, indeed, be harmful. Since it appears that only one substance (coccidioidin) can be extracted from *Coccidioides* cultures, and this substance is an incomplete "antigen", incapable of demonstrably evoking any change in the allergic or immunologic status of *normal* persons* it must be seriously doubted if it can do so in the *infected* individual. It also seems almost inescapable to conclude that the number of

*It must be especially pointed out again that injections of coccidioidin cannot induce the ability to develop the delayed tuberculin type of cutaneous reactivity in normal individuals, i.e., the very type of reactivity which serves the clinician as a rough measuring stick in estimating the degree of the patient's immunity

organisms thriving in the tissues of the seriously involved host must be already producing an amount of this same material (coccidioidin) far exceeding the tiny amounts advised for treatment purposes. It will be at once evident that the antigenic substance or substances formed in the human body by *Coccidioides* organisms *actively engaged in producing the disease* certainly differ from the coccidioidin extracted from cultures. At least it is true that *efficient immunity* develops under such circumstances in most persons whereas it cannot be induced by injections of coccidioidin. Whatever these substances may be, or whatever methods other than the production of "substances" may be involved in the stimulation of the development of immunity, it is difficult to believe that these same activities *on the part of the fungus* are not carried on in identical fashion in the patient who does *not* succeed in developing immunity as well as in the one who *does*. The advocates of "vaccinotherapy" of this sort would have us believe that tiny quantities of a substance demonstrably *inefficient* in pertinent features, can induce the development of immunity in *infected* persons who have previously failed to do so, even though their diseased tissues contain organisms actively engaged in producing the very set of substances or conditions which are *known* to be efficient in the vast majority of *other* infected individuals. Even if a practical method of culturing the organism in the pathogenic tissue phase (spherules) were to be developed and an extract made therefrom, the prospect of such material proving efficient as a vaccine seems therefore to be discouraging. Thus, it seems certain that *failure* to develop immunologic resistance is due to an inherent defect on the part of the *host's* abilities, rather than to a deficiency of the *activities of the fungus*.

Is "Vaccinotherapy" Harmful? Conversely, it *has* been shown that the degree of reactivity of the delayed tu-

berculin type to the intracutaneous injection of coccidioidin can be lowered by repeated injections of increasing quantities of this material (desensitization). This is also significantly true apparently of other fungus extractives such as blastomycin, histoplasmin and trichophytin, as well as tuberculin and many other substances. Recalling that those very persons exhibiting the *highest* degree of such reactivity will prove to be best able to resist the disease, it is apparent that any significant lowering of such ability to react (desensitization) might be logically expected to *lower* the patient's power of specific resistance to a corresponding degree. This author therefore believes that the administration of coccidioidin as a "vaccine" is more likely to be a step in the wrong than in the right direction, even though the use of such small quantities of material as are usually advocated probably do not constitute a very large "step."

Dr. Harry P. Jacobson of Los Angeles was the earliest advocate of vaccinotherapy in coccidioidomycosis, and he continued to believe in its efficacy until his death in 1952. On several occasions at medical meetings, Dr. Jacobson presented a number of patients who had been "cured" after having had the disease in such extensive, serious form that all clinical experience would have led death to be certainly expected; among these were some deeply pigmented Negroes, who are known to resist the disease very poorly. Up to a decade ago this author was highly impressed, as were many other students of the disease, with the value of this therapy. During the succeeding years, however, further study together with continued observations of Jacobson's method in actual use have almost completely reversed this opinion Disseminated coccidioidomycosis occurs in all gradations of extent and severity; and, under widely varying routines of treatment.

approximately half of the patients eventually recover completely. Among these there are always some whose clinical condition at some stage of their disease warranted a fatal prognosis. It has been pointed out that a prediction of death based on clinical grounds alone is not accurate if the interplay between the skin test reactivity and the complement fixation reaction titer persistently points toward recovery. Dr. Jacobson did not accept these tests as of value in prognosis, yet he utilized the intracutaneous reaction as a guide to the initiation of his vaccinotherapy, withholding the vaccine unless and until the patient began to *show* such reactivity. Since this very *sign* is believed to be the earliest indication that the patient is *beginning* to develop a significant degree of immunologic resistance, it is difficult to escape the conclusion that Jacobson's series of patients treated by vaccinotherapy automatically included only those whose prognosis was considerably *better* than the poorest. Also, since complement fixation tests were not obtained at sufficiently frequent intervals for prognostic curves to be drawn, it is impossible to evaluate his statistics accurately. *Before the value of a treatment method can be determined, it is necessary to be able to predict what will happen to patients without it, or to include a parallel untreated series as controls.* This author was much impressed by his experience in caring for one deeply pigmented man, for whom Jacobson's vaccine was sought because of a hopeless prognosis. A combination of circumstances, considered to be unfortunate at the time, made this unfeasible, thereby cheating vaccinotherapy out of another "cure," for soon thereafter the patient began to improve spontaneously, and eventually recovered completely.

Recent correspondence with Stewart reveals that he agrees that the "soluble specific substance" of Hirsch,

Benson and D'Andrea is a polysaccharide complex haptene and not a complete antigen in its own right. This substance however does not include everything produced by *Coccidioides* in culture since it has been purified and heated, thereby destroying at *least* its power to enter into the complement fixation reaction. Stewart concentrated his activities in searching for a *protein* component, and utilized Krueger's method of grinding the culture mat in a ball mill to release all possible substances without "denaturing" them by chemicals or by heat. Because of apprehension over the chance of laboratory infections most of this work was carried out on *Trichophyton* species, from which (to quote Stewart) "good undenatured fungus antigens were obtained which were able to produce antibodies when inoculated into animals and yielding complement fixation and precipitin tests from their sera." In using this material to test human beings, good skin reactions were obtained and desensitization could be demonstrated. Stewart also states that "if I were still active in coccodioidomycosis research I would use an undenatured coccidioidin intradermally, rather than intravenously since I think it is by far the better for immunization and desensitization."

Two discrepancies seem important in carefully reviewing the above statements. First, the antibodies produced in animals were those giving the ability to enter into the complement fixation and precipitin reactions only, and it has been shown that the possession of such antibodies as *these* by a human being certainly does not confer immunity. Stewart does not mention having succeeded in inducing skin test reactivity of the delayed tuberculin type, which is the only type of reactivity which even *appears* to be related to immunity. Second, the fact that his material was capable of acting as a skin testing material yielding positive reactions and producing desen

sitization in human beings does not demonstrate that it contained any extra, vitally important ingredient over the "specific soluble substance" of Hirsch *et al.* which will perform these reactions equally well and which *still* fails to act as a complete antigen able to produce the ability to so react in a normal individual.

Once again it is necessary to emphasize that the mere production of "*antibodies*" does not necessarily mean that the animal thereby acquires any immunologic resistance. It is necessary that these antibodies be the *right* ones, that is, those specifically involved in the immunity processes.

At this writing in would appear that attempts a specific vaccinotherapy should be made only if some loopholes in the above thesis are discovered and logically seem likely to afford success.

Surgical Intervention

Surgical intervention is frequently employed in treat-g coccidioidomycosis, usually consisting either of lobec-ny or pneumonectomy to control persistent cavities in the primary pulmonary form of the disease, or simila procedures (as well as amputation of limbs) for the disseminated, granulomatous type Although it must be admitted that a study of the literature indicates that a large percentage of patients improve *after* such operations, it is difficult to ascertain from the details included in the case histories how many of them would have improved equally well without such intervention Many of the reports do not include repeated complement fixation titers nor quantitated intradermal tests by which curves could be constructed to predict the prognosis to be expected if surgery were not employed Neither are there adequate analyses of comparative groups of unoperated cases to serve as controls The attitude of some surgeons is best understood

from the statements of Cotton and Birsner: "we disregard all laboratory findings and view the complications of pulmonary coccidioidomycosis from the mechanical standpoint only"; and "suitability for operation can be determined without regard to the etiologic nature of the pathologic condition or whether the disease is in a dormant or active phase"; and "only in determining the priority for the patient on the operating room schedule is the medical status of the disease significant." Several of their patients exhibited preoperatively positive reactions to the skin test while the complement fixation test was negative, indicating a good prognosis at that moment. *All* of their patients who presented *positive* complement fixation reactions also showed *positive* skin test reactivity, a picture still far more worthy of optimism than that shown by those destined soon to die of the disease. No complement fixation titers are recorded, but one patient is said to have shown a "high titer bordering upon dissemination" and another "in the range of dissemination." As has been stated earlier this level is approximately a 1-32 dilution. When it is recalled that in *seriously* ill patients titers have been observed as high as 1-4,096 before death ensued, the prognosis in even these two cases, obviously the worst in the series, could not have been considered *really* poor.

Coccidioidal pulmonary cavitation is not often an indication for surgery in the opinion of Winn. Reporting on 92 cavitation cases, he observed that two-thirds healed with medical treatment alone, pneumothorax cured 18 more, and lobectomy or resection was resorted to in 14. Winn states that he has "observed no instance of fatal dissemination from a residual cavity, and this possibility is not to be considered as an indication justifying surgery." Farness, who was the first to observe pulmonary cavitation in coccidioidomycosis (1932) said in 1951 "although exci

sional surgical procedures seem without danger in spreading the infection, a word of caution is in order to the overly-eager. We know of such patients in whom new cavities developed in the expanded lung." Also, "cavitation is no indication of dissemination. These patients are generally not handicapped, and a reasonable waiting period should be allowed before adding the hazard of collapse therapy or surgical intervention." When it is noted that among the patients reported by Cotton and Birsner were some who were able to return to work as early as 3 weeks after lobectomy or pneumonectomy, and the rest within 2 months, it must be concluded that they could not have been very severely debilitated before surgery.

The full significance of the prognostic value of the skin test and complement fixation titer should be evaluated before surgery is decided upon As long as they furnish a good prognosis, and the curves determined by their repetition at monthly intervals indicate that progress toward recovery is being made at a satisfactory rate, surgery should be withheld. If a few such successive months of observation indicate no progress, it should then be asked whether the disease is sufficiently well localized to allow the *total* of involved tissue to be surgically removed. Especially valuable in this regard will be the complement fixation titer, since it may be higher than could logically be attributed to the disease if confined to the area proposed to be removed surgically, indicating the presence of extensive lesions elsewhere. If this titer exceeds 1-32 it is likely that the diagnosis is no longer "primary pulmonary coccidioidomycosis" of severe degree, but the "disseminated" form in which lesions elsewhere than in the lung are probably *already* present. If these extra pulmonary lesions cannot be located by symptoms, physical ex-

amination or radiography, their presence in "silent" areas must be considered likely and their extent can be best estimated by the height of the complement fixation titer.

The question of surgical intervention in cases of disseminated coccidioidomycosis apparently localized to a single limb should also be subjected to the same analysis. The complement fixation titer may be incompatibly high to support the contention that the disease is actually so localized as to be subject to complete extirpation; conversely, the prognostic curves furnished by repeated skin and complement fixation titers may indicate that complete recovery is to be expected without surgery, thereby saving a useful limb.

Chemotherapy

At the present writing, no drug has been proved to be of specific value in coccidioidomycosis, and some are almost certainly harmful. In the primary pulmonary type, it is probably wise to withhold trial of all such measures, since the chance of spontaneous complete recovery is so overwhelmingly great that there is more likelihood of interfering with this important natural process in some unpredictable manner, than of assisting the patient. In the disseminated type, Cohen and Bos (1952) summarized the status of ten drugs selected because of their ability to inhibit the growth of C. immitis in culture. By local application, sodium caprylate was classed as useful, actidione and fradicin as of slight benefit. By systemic administration, actidione was said to be slightly successful but too toxic, ethyl vanillate encouraging, protoanemonin and sodium caprylate ineffective, and fradicin too toxic. Prodigiosin was reported by Wier, *et al* as encouraging; of 14 cases, the disease was arrested in 3, improvement was noted in 3 more while 8 did not respond. These percentages do not indicate a much higher degree of success than

other methods of therapy. This author has seen cases which failed to be benefitted by stilbamidine, stilbestrol, ethyl vanillate and delta-5-pregnenolone. Sulfonamides and iodides are ineffective. Gentian violet, thymol, and heavy metals such as arsphenamine, colloidal copper, and antimony have been long ago discarded. Rimocidin and thiolutin have not been furnished in injectable form. Some of the newer derivatives of stilbene or nitrostyrene being investigated by Curtis, Harrell and Bocobo for blastomycosis may perhaps warrant trial. A survey of the most recent literature should precede any such attempts. The most recent promising medicament is an antibiotic, mycostatin. Said to be effective in animal infections and cautiously being used for severely ill patients, this material is to be watched expectantly.

Since corticoid therapy tends to abolish or reduce the intensity of allergic reactions it was anticipated that it might also interfere with the development of immunity in coccidioidomycosis; particularly was this predicted by those who believe the two processes are closely related. Several patients are known to have received such drugs without benefit and in most instances they were rendered worse. Animal experiments indicated that this form of therapy is distinctly harmful (Sternberg, Tarbet and Wright). Further trials are probably inadvisable, indeed such drugs should be specifically interdicted except perhaps in quantities small enough to *mildly* ameliorate a *severe* erythema-nodosum-multiforme "id" reaction.

Antibacterial antibiotics apparently cannot be of benefit, and on several occasions have appeared to have been harmful, possibly by interfering with the proper digestion and absorption of proteins and of components of vitamin B. Also they may furnish "antigens" of fungus origin capable of interfering with the development of immunity,

or "desensitizing" the patient to his skin test reactivity. They should be withheld unless indicated by specifically demonstrable secondary bacterial complications.

The discouraging aspect of the therapeutic methods so far discussed should explain the present view that the most beneficial feature in the treatment of disseminated coccidioidomycosis is prolonged bed rest until the prognosis is established as good, not only by physical examination, freedom of symptoms and favorable laboratory findings, but *also by the interplay between the intradermal reactivity and the complement fixation titer.* Too early resumption of physical activity has been observed to be disastrous on many occasions, but it still occurs all too frequently, especially in cases under the supervision of those who do not place reliance in the significance of these extremely valuable prognostic tools.

Since antibodies are protein molecules, and on the basis of the theory that they play a part in developing immunity, it seems advisable to prescribe a diet high in proteins. This also tends to prevent the wasting away of the body's vitality in general. Several components of vitamin B are thought to be intimately concerned with antibody formation and the development of immunity. Riboflavin, pyridoxine and niacin are apparently among the most important Also vitamin C must be considered helpful.

The possible specific benefits to be derived from multiple small transfusions of whole blood have been discussed in the paragraphs discussing passive immunization.

Chapter 8

SPOROTRICHOSIS—HISTORY AND THE CLINICAL SYNDROMES

In the present volume, as was explained in the Introduction, the subject of coccidioidomycosis has been treated first and in extensive detail, not because it is inherently more important than the other mycosis, for of course it is not, but in order that some of its phenomena which are more well defined and easily interpreted, can serve as a foundation for instructive comparisons with the other diseases as they subsequently are brought up for discussion. For essentially the same reason sporotrichosis is taken up next, again not because of importance, but because it too possesses some well established characteristics, in this case some which are particularly well adapted for the first of such a series of analytical comparative studies. In these, the various fungous diseases will be compared with one another in an attempt to delineate some clues which appear to be valuable assets in bettering our understanding of the mycoses in particular and infectious diseases in general. Before embarking on this course, however, it is necessary first to present our *factual* knowledge of clinical sporotrichosis.

Sporotrichosis is an infectious disease which develops in man and animals as the result of the acquisition from an exogenous source in nature of spores of the fungus *Sporotrichum schenckii* The organism enter almost invariably

through a break in the skin such as a scratch or a puncture wound. A syndrome develops subsequently which resembles so closely the primary stage of cutaneously acquired syphilis that it is termed "chancriform." Much more rarely a generalized form of infection occurs.

Schenk's first report in 1898 was sufficiently thorough to establish the clinical picture almost as completely as it exists today. In a series of important papers beginning in 1903, De Buermann, collaborating with Ramond, Gougerot and others, contributed many facts about the fungus, and in addition described the uncommon *generalized* type of the infection. In 1910 when more than a hundred cases had been reported, Hyde and Davis published an extensive review with more than 150 references.

Pijper and Pullinger in 1927 reported 14 cases resulting from injuries sustained by native workers by contact with the timbers in South African mines. This was the beginning of a surprisingly extensive epidemic, totalling almost 3000 cases by 1944 (Dangerfield and Gear, Helm and Berman). An extremely valuable study covering this epidemic was published as a monograph by the Transvaal Mine Medical Officers Association in 1947.

Although there is considerable variation in the morphology of various strains, it is usually accepted that there is but one species of fungus involved, called *Sporotrichum schenckii*. This organism has been found widely distributed in nature especially as a saprophyte on vegetation, living or dead, and on animal excreta. Human cases occurring sporadically have been traced most frequently to the thorns of the barberry shrub (Foerster) sphaghum moss (Gastineau, Spolyer and Haynes) or hay (Singer and Muncie). It has not been possible to define any geographic limitations, and cases have been reported from all areas of the globe where there is personnel capable

of making the diagnosis. There are certain circumstances which apparently enhance the growth of the fungus in a particular environment, for example Brown's discovery that it grew on mine timbers only where the relative humidity was between 92 to 100% and the temperature between 79° and 81° F.

No age is exempt and there appears to be no variation in susceptibility because of race. There is a definite predominance in males, almost certainly attributable to the increased likelihood of cutaneous injury during work or play. That local conditions can reverse this trend however was shown by Padilha-Gonçalves and Peryassu, who observed in Brazil a higher incidence in females.

In their extensive writings, De Buermann and Gougerot classified sporotrichosis into six clinical types (lymphatic, disseminated, epidermal, mucosal, skeletal, and visceral) although many of these cases presented lesions belonging in more than one of these groups. Such a multiplicity of types seems unnecessary, particularly since lesions of all of these classes in various combinations, have also been long recognized in most of the other deep mycoses, in none of which has it been found desirable to consider them as six separate clinical entities. The localized type of sporotrichosis resulting from direct primary inoculation into the skin is certainly distinct, as is the disseminated form; most of the other classes are probably variants of one or the other of these, and will be so treated here.

Primary Cutaneous Sporotrichosis

Primary cutaneous sporotrichosis results when *Sporotrichum schenckii* is inoculated directly into the skin of a previously uninfected person. Almost always this is accomplished by an injury sustained by contact with wooden splinters, thorns or other vegetation. L. M. Smith

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accepted a lesion on the cheek of an infant as having occurred directly from the infected cheek of the mother, the only instance of transference from human to human or from animal to man. Sometimes, however, there is no history of cutaneous injury, and penetration through the walls of intact hair follicles has been considered probable.

After an incubation period of from 3 to 21 days (sometimes apparently prolonged as much as several months) an elevated papule appears, pink or cyanotic in color, frequently oval in shape with its long axis directed in line with the natural skin creases. In 60% of the 2,825 cases studied by Helm and Berman, the initial lesion was on the hand or arm, on the trunk in 23%, on the legs in 11%, on the face or neck in 2%, and in the remainder multiple lesions were present. However in *this* series it was evident that the principal reason why other areas were not involved was because of the protection afforded by the type of clothing worn by the native miners.

It is remarkable that in most instances the primary lesion is not painful unless secondary bacterial infection is also present, also, there is usually but little exudate. The papule slowly enlarges and begins to ulcerate in the center, presenting eventually a crater with a bright red base free of necrotic tissue, and with ragged, undermined edges, which bleed easily when traumatized. Frequently *similar small secondary papules appear at the periphery.*

After a week or more, most patients begin to develop nodules along the lymphatic channels which drain the area. These are small and demonstrably subcutaneous at *first*, but rapidly enlarge and soon become attached to the skin. It is surprising to find sometimes that the nodule farthest from the original chancre is the most advanced. There is also lymphangitis in these vessels. The nodules frequently *ulcerate until they appear* as miniatures of the

parent chancriform lesion. The regional lymph nodes become enlarged but usually do not ulcerate. The general health of the patient is not affected as a rule, but there is little tendency to *complete* spontaneous cure.

Although this lymphatic involvement is typical, in some cases it is minimal or even absent, and in these the disease spreads in the skin itself, forming a flat placque, sometimes smooth, sometimes covered with silvery scales. A warty form is occasionally seen, most often on forehead or knee. Sometimes these warty-growths become extensive and are accompanied by pustules, simulating then verrucous tuberculosis or blastomycosis. (Smith and Garrett). Such variations as these are thought to be due to factors which are but little understood as yet, such as local tissue resistance, specific or nonspecific general body resistance, and perhaps the depth, quantity and virulence of the original inoculation. Experimental infections in human beings are said by Helm and Berman to suggest that *intradermal* inoculation may produce the mild superficial placque type, while the subcutaneous route produces the ulcerative and lymphatic form

Sporotrichosis has been reported as beginning in the mucous membranes of the nose, mouth or pharynx. Sometimes the clinical picture subsequently appears chancriform and results in regional lymphatic involvement in a manner strongly suggesting that the primary inoculation occurred through the mucous membranes in a manner analagous to the primary cutaneous form. Other cases seem to indicate that such lesions originated by dissemination from *within*, since other internal organs are *simultaneously* found to be involved

Non-tegumentary Primary Sporotrichosis

It must be considered highly significant that in the series of almost 3,000 cases occurring in the African mines

in which all evidence indicates that the infection occurred by *intracutaneous primary inoculation, no case of dissemination* to other organs was encountered. This aspect will be discussed more fully later (page 90).

If the concept indicated by this trend is correct, and we conclude that only the chancriform syndrome without systemic dissemination can develop from a direct *cutaneous* primary inoculation, it seems necessary to postulate a *non-tegumentary* portal of entry to explain the rare disseminated form soon to be discussed. Conclusive proof of this is difficult to obtain. De Buermann demonstrated that *S. schenckii* could pass unaffected through the intestinal mucosa when ingested on contaminated raw fruits or vegetables, but cases exhibiting lesions of the gastrointestinal tract are very rare. Simson reported that the infectivity of cultures was retained, although in lessened degree, after 2 years of drying, and the fungus was recovered once on a plate exposed to air currents in a mine shaft, indicating at least a *possibility* that the infection could be acquired by *inhalation*.

However it may *not* be necessary to have a non-tegumentary portal of entry. It may be that the correct answer is that thought to apply to coccidioidomycosis, where dissemination appears to occur only in a very small percentage of infected persons (about one per thousand), and because these individuals possess some defect in their immunity mechanisms, even perhaps before the inoculation occurred. In coccidioidomycosis it seems likely that this percentage would be similar in both the primary *pulmonary* type and in the primary cutaneous form, although it has not as yet been conclusively demonstrated in the latter. However, if this *is* the true situation, it would seem likely that at least a case or two of dissemination would have been discovered in almost 3,000 cases of sporo-

trichosis. Awaiting further data, at present it is possible to consider this entity only vaguely, as is done here, and pass on to what is probably its sequel, the disseminated type.

Disseminated Sporotrichosis

First it is necessary to differentiate "dissemination" from the admittedly rather extensive spread of the typical intracutaneously acquired chancriform lesions into the regional lymphatic structures. In contrast to this, by the term "dissemination" is meant the scattering of the infection widely over areas of the body far distant from the primary lesion by organisms transported by means of the blood stream, as is typically observed in coccidioidomycosis.

Disseminated sporotrichosis has been reported but rarely, although it is likely that additional cases exist which were never correctly diagnosed. The majority of the reported cases occurred in France in the earlier years, but sporotrichosis has completely disappeared there in the last 10 years (Drouhet). The writings of De Beurmann are the best source of information. Foerster, Moore and Kile, Collins, and Cawley have recorded this type in America, and Gonzalez Ochoa in Mexico. The manner in which the infection was originally acquired has usually been obscure, but enough instances of involvement of the mouth, pharynx or lungs have been observed to suggest that these routes are perhaps the likely ones

In its most common form, disseminated sporotrichosis becomes apparent as multiple subcutaneous nodular masses scattered over the body, which soften, and after incision or traumatic rupture, form chronic ulcers. From these the infection tends to spread to involve larger areas of the surrounding skin, simulating the lesions of tertiary syphilis, tuberculosis or the other deep mycoses Spon-

taneous cure is rare, and response to treatment has been recorded as poor but somewhat variable. Some cases progress rapidly to death, while others remain chronic for months.

There is often involvement of other parts of the body, such as *bones, joints, muscles, tendon sheaths, lungs,* genitourinary system or other viscera sometimes in conjunction with the cutaneous abscesses, sometimes without them.

Pathology

The pathologic tissue changes in primary cutaneous sporotrichotic lesions in human beings have one outstanding characteristic, the causative organisms are almost never recognizable as such (if indeed they are visible at all) in tissue sections or exudates, even with the periodic-acid-Schiff staining procedure so useful in other fungous infections. This is surprising, since they are easily defined in tissues from experimentally infected animals and in those from the viscera of human beings possessing the disseminated type. They are tiny, cigar-shaped bodies, 3 to 5 microns in length which bear from one to three small oval buds at either or both poles. It is possible that they are actually present in the chancriform type, in this form (or in a "coccoid" stage perhaps) but that they remain undiscovered because they are too small, too sparsely distributed or too poorly defined to be differentiated from cellular debris. Occasionally a larger "asteroid" form may be seen and lead to the diagnosis. It may also be that the organisms are present in larger numbers immediately after the inoculation, but are soon diluted and decreased in actual numbers by the intense defense mechanisms of the host. Dr. Lisboã Miranda of Rio de Janeiro recently showed me a section from human chancriform sporo-

trichosis in which a fair number of easily identifiable organisms were to be seen.

A sporotrichotic nodule usually shows several more or less concentric zones, the central one composed of an anuclear mass of necrotic material, sometimes purulent, surrounded by polymorphonuclear leucocytes, merging into a zone of fibroblasts, lymphocytes, epithelioid cells and giant cells. Except for this tendency to concentric zones the granulomatous picture cannot be differentiated from syphilis, tuberculosis or other deep mycoses.

Fortunately, cultural methods are usually successful in easily demonstrating the presence of *Sporotrichum schenckii* in any stage of the disease. The fungus is easily isolated in culture, grows well and is readily identified. The use of the skin test and complement fixation reactions in diagnosis will be discussed in Chapter 10.

Chapter 9

SPOROTRICHOSIS—THE IMPORTANCE AND SIGNIFICANCE OF THE PORTAL OF INOCULATION AND THE CHANCRIFORM SYNDROME

In the past it has been customary to emphasize the fact that each of the infections in the category known as the "deep mycoses" presents a clinical syndrome differing markedly from the others, the total of these differences conferring upon the group as a whole the ability to duplicate almost any combination of signs and symptoms found in chronic disease. There are indeed important differences, some of which are undoubtedly attributable to certain inherent preferences on the part of the causative fungi for special environmental features. For example, the deep seated nature of actinomycosis is, in part at least, a reflection of the preference of the causative organism for anerobicity. The persistent refusal of viruses to grow outside of living cells makes it easy for us to accept the fact that *Histoplasma capsulatum* has a similar proclivity, and it occasions no great surprise to find that *Cryptococcus neoformans* has a predilection for nervous tissue. That the diseases which these organisms cause should consequently differ considerably one from another is altogether to be expected.

However, it is at least equally important to emphasize certain fundamental *similarities* in the manifestations of

the mycoses, for by discovering wherein *some* are *alike* it may be possible to discern certain basic patterns to which they might *all* be found to adhere if our knowledge were sufficiently complete.

Certainly it is obvious at a glance that in neither of its common forms (the primary pulmonary and the disseminated) does coccidioidomycosis resemble in the least the *ordinary* type of sporotrichosis; they are as different, perhaps, as those of any other pair of chronic diseases which could be selected. Coccidioidomycosis is acquired by nearly all persons living in certain regions, but so mildly that most of them remain entirely unaware of its presence while a spontaneous cure occurs, conferring subsequent immunity to reinfection; most of the remainder also achieve the same result but only after a variable amount of clinical illness centering about the lungs; while a very few sustain a widespread disseminated chronic granulomatous disease highly dangerous to life itself. Conversely, sporotrichosis typically results in a chancriform syndrome, represented by an initial ulcerative papule accompanied by acute lymphangitis, nodules along the lymphatic vessels draining that area and, regional lymphadenitis; the entire process usually remains limited to the affected limb to the virtual exclusion of dissemination into a fatal form.

It is not generally realized, however, that there is an entirely logical reason for such a marked difference in clinical appearance in the fact that each of these two infections is almost exclusively acquired in a manner entirely different from the other, through a different portal of entry, and involving a different tissue primarily. It has long been recognized that coccidioidomycosis is almost *always* acquired by the *inhalation* of the arthrospores of the fungus along with wind-borne dust, resulting in a primary *pulmonary* infection, whereas sporotrichosis is

equally predominantly acquired by the *inoculation* of the fungus through the *skin* by a puncture wound. It is at once obvious that with such a difference in the potrals of entry, the *typical* or "*common*" forms of the two disorders could not be expected to fail to differ accordingly. While *other factors may also be somewhat influential, it is not necessary* to invoke them to explain adequately this phenomenon.

Having disposed for the moment of the typical forms of these two diseases, let us now consider whether or not a variation in the portal of entry can account also for their more *unusual* manifestations. What happens if these routes of infection are reversed? First, what is the result of intracutaneous inoculation of *Coccidioides immitis?* Throughout the years there have been a few cases of coccidioidomycosis in which it first appeared as a lesion of the skin, without evidence of previous pulmonary involvement, usually beginning as a subcutaneous nodule which soon became ulcerative, and then persisted as a slowly enlarging chronic granulomatous placque, without any lymphatic sequelae This was frequently followed by other similar lesions elsewhere on the skin. It was usually concluded *that this clinical picture* was due to the fungus having been inoculated directly into the skin at that point although in no recorded instance was this supported by adequate proof, *many such cases lacking even the history* of *any* cutaneous wound *or* injury. Nevertheless, this syndrome came to be considered as the expected result of direct cutaneous *inoculation* of *Coccidioides* (the so-called "primary cutaneous" form) *in* spite of the fact that it *differed almost totally from that known to result* when *Sporotrichum* is similarly inoculated.

In 1918, the author, among others, was privileged to observe and follow the case of a mortuary attendant who.

while preparing a body heavily infected with *Coccidioides* allowed pus to enter a deep abrasion on his finger. Subsequently he presented a clinical picture *in no way* resembling that which previous opinion would have led to be *expected*, but instead entirely typical of that seen in the usual form of *sporotrichosis* (a chancriform primary lesion accompanied by lympadenopathy and nodules), differing only in that *Coccidioides* was cultured and demonstrated histopathologically instead of *Sporotrichum*. (See also under Primary Cutaneous Coccidioidomycosis, page 13) for additional details). He recovered spontaneously and has remained free of recurrence. In 1955 Trimble and Doucette observed a case presenting the identical chancriform syndrome which was also known to have resulted from an intracutaneous inoculation this time in a laboratory worker. While it is admittedly unwarranted to draw conclusions from the observation of only two cases, the fact that these are the *only* instances which resulted from *proved intracutaneous* inoculations should cause them to be given great weight when considered against the evidence assembled in those cases in which such a route was *only surmised*. When in addition it is pointed out that the same type of cutaneous involvement as that previously *supposed* to result from cutaneous inoculation has been observed repeatedly when the lungs were *known* to have been the primary locus it becomes logical to ascribe *all* such cases to a similar mechanism Thus it appears likely that when inoculated directly and primarily into the skin of previously uninfected persons *Coccidioides* and *Sporotrichum* produce identical chancriform syndromes

Conversely, what happens when sporotrichosis is acquired by a route other than its usual cutaneous inoculation? Cases of disseminated sporotrichosis have been occasionally observed, the clinical picture bears many simi-

larities to disseminated coccidioidomycosis, exhibiting multiple subcutaneous nodules and abscesses and verrucous plaques, as well as pulmonary, bone and visceral involvement; and terminating frequently in death. *No conclusive evidence is at hand which would indicate that this* syndrome has ever developed from the *ordinary* chancriform type of sporotrichosis; indeed the mode of entry of the fungus in these cases has usually been inapparent, but sometimes *presumed* to have been either by inhalation or ingestion. That this syndrome probably *never* results from primary *cutaneous* inoculation may be concluded from the fact that it was not observed *at all* in the series of 2,875 cases occurring in the South African mines under conditions in which the skin was almost certainly the only portal of entry. Also, in none of the American cases of disseminated sporotrichosis reviewed by Collins was the portal of entry *known* to have been the skin. Likewise in France, where the disseminated form occurs more frequently, a proved primary cutaneous lesion has not been observed to precede it. Since the reverse appears to be even more unlikely if not impossible (that it, for a chan*criform syndrome to result from an inoculation elsewhere* than into the skin), we may logically conclude tentatively that when *Sporotrichum* infects the human body by a route *other than the skin*, the resulting syndrome resembles *disseminated coccidioidomycosis* much more than it does the *conventional* type of *sporotrichosis*.

It is thus evident that *most*, if not all, of the extensive *apparent* variations between coccidioidomycosis and sporotrichosis may be explained on the basis of the *difference* in the *portal* of *entry*, and that when this influence has *been appropriately evaluated* the two diseases are closely similar. It is indeed surprising how *little* variation remains unexplained by this thesis, especially when it is

recalled that in the infected tissues the two fungi bear no resemblance to each other and call forth entirely different histopathologic responses. It would, indeed, be strange if the diseases resulting from such dissimilar organisms were *entirely* alike.

It is interesting to explore the reasons why *Coccidioides* and *Sporotrichum* each assiduously selects a different portal of entry, almost to the exclusion of the other. In artificial culture at ordinary atmospheric temperatures (conditions under which these two fungi maintain themselves in their natural reservoirs), they exhibit characteristics which appear to explain adequately the above variation in their selection.

Coccidioides immitis grows as a fluffy, filamentous colony resembling cobwebs, much of which is composed of specialized hyphae segmented by fragile cross walls into arthrospores with a narrow collarette between them. These spores break apart, one from another, with extreme ease; allowing the smallest air current to detach a cloud of them from the surface of the colony. They are so light that they are easily borne on the wind for great distances. By the same token these infectious elements cannot cling very tenaciously to the surface of materials capable of inflicting puncture wounds into the skin so that no large numbers of them could be implanted deeply by such a means. It is therefore *entirely logical* to find that this infection is almost always acquired by the inhalation of the fungus, to the virtual exclusion of the intracutaneous inoculation route.

Conversely, *Sporotrichum schenckii* grows typically as a moist mat of densely interwoven filaments closely applied to the surface of the medium, preferring, in nature, materials capable of furnishing thorns and splinters such as vegetation and wood, into whose crevices it ex-

tends itself, and presenting no dry aerial cottony elements which air currents might easily remove. It is admittedly true that the spores are attached to the hyphae by fragile thread-like sterigmata, so that if the entire culture and its substrate be dried, such spores may become windborne. This must happen but rarely however; for example, in an environment where it was present abundantly growing on wooden timbers, *Sporotrichum* was recovered on culture plates exposed to *air currents* only once out of many attempts. From these considerations it is easy to understand the almost invariable entry of this fungus into the human body by way of a cutaneous puncture wound, with only an *occasional* instance of pulmonary invasion.

If a new theory is actually correct, additional support can frequently be obtained by testing its ability to clarify similar phenomena when applied to other diseases. During the discussion of North American blastomycosis soon to follow it will be shown that when *this* fungous disease is acquired by *primary inoculation* into the *skin*, a *chancriform* syndrome follows, practically identical to that produced by sporotrichosis (see page 109). Also, histoplasmosis is thought to have been observed behaving similarly (see page 149).

It is also highly significant that the skin test in cases of coccidioidomycosis of the primary cutaneous, chancriform type has revealed a high degree of positivity while the complement fixation reaction was of extremely low titer. This combination of results was accepted by the observers very early in the course of the disease as an indication that it would pursue a benign course, which the subsequent progress of the patients to complete cure has borne out (see page 15). In the two instances of blastomycosis following primary cutaneous inoculation in which such tests were emplo[illegible] same phenomena were re-

vealed, and the same prognosis eventually shown to be correct (see page 110).

All of these observations, clinical as well as mycologic and immunologic indicate that, for the chancriform syndrome to develop, the body of the host must actively resist the infection and its progress to a considerable degree, either by immunologic, allergic or cellular means. This does not mean that such resistance will necessarily be efficient enough to result in *complete* eradication of the infection without the aid of other therapy; in fact in almost all diseases it is *not*, being followed either by a prolongation of the localized lymphatic involvement or by dissemination of the organism to produce secondary lesions elsewhere in the body as in syphilis, yaws and American leishmaniasis. Nevertheless the chancriform syndrome *itself* appears to be an essentially benign process in all those diseases in which it occurs. In those cases *thus far recorded*, of coccidioidomycosis, blastomycosis, and histoplasmosis complete recovery has taken place. Of course, it is to be expected that if such an inoculation were to be sustained by a person who is "*immunologically defective*" (as discussed on page 82) the chancriform process might not follow at *all*, or might be less developed and be followed by *dissemination*. Persons who possess such a defect in the power to develop immunologic resistance to coccidioidomycosis when acquired by the pulmonary route apparently exist in the normal population in a ratio of approximately one or two per thousand. It might thus be expected that if a thousand persons were to acquire coccidioidomycosis by the *intracutaneous route*, one or two might be such immunologic defectives, and might therefore sustain dissemination. This same or a similarly tiny ratio may also exist with regard to sporotrichosis, although it cannot be said to have been conclusively demonstrated

in spite of the fact that in this disease there *have* bee several thousands of persons who have acquired the infe tion by the intracutaneous route. It seems likely, how ever, that such cases have, in fact, existed; and this autho has every expectation of having such a case pointed out t him even though he has as yet been unable to discover on by perusing the literature.

Thus the *primary cutaneous* complexes of cocidioido mycosis, North American blastomycosis, sporotrichosi histoplasmosis, syphilis, tuberculosis, yaws and America leishmaniasis are closely parallel, being in themselves es sentially self limited while in this stage, although in som diseases serious sequelae may follow later.

The chancriform syndrome has not been reported t occur in deep mycotic infections other than the four al ready discussed. However (with the exception of a single instance of South American blastomycosis, see page 181), there have been no cases recorded in which direct cutane ous inoculation of such causative fungi into human be ings has been *conclusively proved*. Lacking experimental procedures in man (or in animals which it is possible might be shown to resist the infections in the same man ner) we can only speculate as to the result of such inocula tions.

However, it would appear unlikely that *Cryptococcus neoformans* could *produce* a *chancriform* syndrome on intracutaneous inoculation because the human body *de monstrably infected* by this organism shows so little abil ity to resist its multiplication by immunologic or cellular means. Also, *this* organism is probably not pathogenic to *entirely normal* persons: it seems to select only those possessing some other disease, usually of lymphoblastoma tous nature, which causes a *defect* in one or more of the mechanisms of *resistance*; characteristics which might be

expected to alter the resulting clinical response. Such persons as these are probably analogous to that small percentage of those exposed to coccidioidomycosis who sustain dissemination and serious sequelae, because of an *inherent defect* in some mechanism of immunity, which in that instance is not as yet defined.

Actinomycosis cannot be compared directly with those diseases observed to yield the chancriform picture since the causative fungus is probably of a very low degree of infectivity unless it is implanted deeply, in large amounts, into traumatized tissues and allied with bacteria. Norcardiosis also presents some of these features, but its immunologic phenomena are even more poorly understood. Both of these organisms are more closely allied to bacteria than are the other pathogenic fungi. The chancriform syndrome is not evident in either disease.

Chromoblastomycosis appears to be acquired almost always by intracutaneous inoculation, yet does not result in a chancriform syndrome. The body seems to have little ability to prevent the spread of the disease, by immunologic means, although the infection remains comparatively superficial and progresses very slowly This fungus is probably not capable of widespread dissemination to produce a fatal result,* and this infection should perhaps thus be placed in a category between the deep and the superficial mycoses. In this present regard it resembles the latter group in not resulting in the chancriform syndrome.

In summary, it seems likely that the intracutaneous inoculation of *any* one of the group of fungi classed as deep mycotic organisms will result in an essentially be-

*There have been a few cases in which dematiaceous (dark-brown) fungi have been observed in tissues of the central nervous system. Some of these were accepted as being caused by the same genera of fungi which cause Chromomycosis (Hormodendrum, Cladosporium, and Phialophora) (Almeida *and others*)

nign chancriform syndrome closely resembling typical sporotrichosis, provided the organism is one which is strongly resisted immunologically or cellularly by the host. The widely disseminated, and chronic cutaneous forms of these diseases probably result only from other portals of entry, principally the lungs or digestive tract even when the first observed lesions are in the skin, and even when trauma *appears* to have played a part. Placing appropriate emphasis upon such similarities as these in diseases which appear at first glance to differ extensively, serves to explain some hitherto perplexing features.

Chapter 10

SPOROTRICHOSIS—ALLERGIC AND IMMUNOLOGIC REACTIONS; THERAPY

BEGINNING in 1908 with the work of Widal and Abrami, and Bloch, extracts derived from cultures of *Sporotrichum schenckii*, termed "sporotrichins" have been employed in testing procedures analagous to those discussed in detail in the chapters on coccidioidomycosis. Most of the reports have emphasized that the results fall short of the goal to be expected of an ideal "antigen," citing examples of "false positive" and "false negative" reactions, so termed because in many instances they seemed to the observers not to follow consistently the clinical status of the patients.

Since the recent publication of reports indicating that when either coccidioidomycosis or North American blastomycosis is acquired by intracutaneous inoculation a chancriform syndrome closely resembling sporotrichosis results, it has been increasingly interesting to compare these diseases in other ways as well. One of the most obvious conclusions derived from such comparisons is that many of the "*discrepancies*" in the results of testing procedures employing sporotrichin are logically explainable if sporotrichosis actually follows the *same immunologic pattern* so clearly visible in *coccidioidomycosis.* Stated differently, if attempts to force sporotrichin testing to conform to preconceived patterns held by the *observers* are avoided

and the tests allowed to *speak for themselves*, then it is logical to conclude that sporotrichosis closely *duplicates* the same phenomena established in coccidioidomycosis.

The Intracutaneous Sporotrichin Test

It is amply evident that the human body strongly resists the intracutaneous inoculation of *Sporotrichum schenckii*, for it produces an intense cellular response both locally and throughout the adjacent lymphatic structures, sufficiently effective to prevent the spread of the infection beyond the inoculated limb, although usually not enough to achieve *complete* spontaneous cure. It will be recalled that when persons suffering from *coccidioidomycosis* exhibit a similar high degree of resistance, it is accompanied invariably by the ability to react strongly to the specific skin test (yielding the delayed tuberculin type of response).* Thus it is to be expected that high skin test reactivity to sporotrichin will also be the rule in primary cutaneous sporotrichosis. Such is indeed the case, de Buermann being the first of many observers to accept a negative skin test as sufficient evidence to *rule out* present or past sporotrichosis. This is reliable in all but the earliest stages, since in infections experimentally produced in a volunteer it has been observed that the skin test became positive as early as the fifth day following the inoculation. Allergic cutaneous reactions ("sporotrichids") have been occasionally observed by de Buermann in hypersensitive individuals, analagous to the "coccidioidids" described on page 11 although much less marked in degree.

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*Gonzalez Ochoa has remarked that the immediate flare reaction has been frequently observed but that it could not be correlated in any way with the presence or absence of the disease nor with its stage or progress in any manner. This is the exact situation observed by most workers in coccidioidomycosis (see page 58)

De Buermann, however, long ago reported "false positive" reactors to sporotrichin, that is, positive reactions occurring in persons having no history suggestive of that disease. In this regard it is important to recall that it is now accepted that in *coccidioidomycosis,* reactions to intracutaneous testing with coccidioidin of the type previously considered to be "false positives" are in reality "true positives," since they occur almost exclusively in persons who have actually *had* the disease and *recovered, even though it may have never been perceptible clinically* It appears at least plausible that the same phenomenon may account for "false positive" reactors to *sporotrichin,* that is that many if not all of these patients actually have previously *had* the disease. Also, Helm and Berman have observed cases of sporotrichosis of a mild, superficial type which healed spontaneously It is easy to realize that in such mild cases as these the diagnosis of sporotrichosis might very easily be *missed,* furnishing thereafter a person who might be later shown to react positively to the sporotrichin skin test and be classed wrongly as a "false positive" reactor. These investigators were also unable to produce sporotrichosis in some persons by experimentally injecting the organisms, indicating the presence of immunity; they do not state whether these persons were tested with sporotrichin, but it might be that they would have reacted positively, indicating that the immunity was of the specific *acquired* type rather than *natural.*

Also, some of the tendency to produce "false" positive reactions may be due to nonspecific protein-like substances or chemical groupings contained with the active principle of sporotrichin It is interesting to note that the sporotrichin used by Norden was sterilized by heating, for it will be recalled that *coccidioidin* may be freed from some of its nitrogen content by such heating without destroying

its power to elicit reliable skin tests. With less nitrogen content, there is less likelihood of "false positive" reactions to adventitious protein-like substances; the *ideal skin testing material* perhaps would be a completely *nitrogen-free* (therefore *protein* free) polysaccharide. Thus it is probable that heating (or other physical or chemical treatment) *should be experimentally employed* in the production of sporotrichin to eliminate as *much nitrogen as possible* before it is concluded that its tendency towards false positive nonspecificity *cannot be reduced.* Gonzalez Ochoa has recently prepared such a "purified polysaccharide" from *Sporotrichum schenckii* which he believes to be very highly specific in this regard. Although his material still contained 8% of nitrogen it did not give positive Millon, biuret or xantho proteic acid tests, indicating the lack of protein in *chemically* significant amounts, but it *might not be immunologically protein free, since* immunologic reactions constitute a much more delicate testing procedure.

Apparently patients who have had sporotrichosis in the severe disseminated form leading to fatality have not been adequately studied with regard to sporotrichin testing procedures. Experience with coccidioidin would lead *us to expect that such persons would react poorly, if at all,* to the intracutaneous test, at least for a varying period before death, furnishing thereby examples conflicting with the opinion that such a negative reaction "rules out" sporotrichosis (which it apparently *does* reliably in the ordinary chancriform type). It is likely that this state of affairs has arisen simply because of the extreme *rarity* with which fatal disseminated sporotrichosis occurs, *when* compared with the *frequency* of the primary cutaneous nonfatal form. In the series of cases reported by Gonzalez *Ochoa, one patient who had sporotrichosis generalized*

through the blood stream failed to react to the skin test. It will be interesting to watch for the results of sporotrichin testing in future reports of such cases.

The Complement Fixation Test

The complement fixation test has given "variable" results in the hand of several investigators. Norden found it to be positive in but 2 of his 11 cases, the titer not being specified. Again, this is not surprising if this reaction adheres to the pattern which seems to be established in coccidioidomycosis (and probably in both of the blastomycoses and histoplasmosis as well) in which the complement fixation *titer* parallels the *total quantity* of *involved tissue* (or in a later view, the *total number* of *organisms* actively engaged in producing the disease) at the time the test is made. In the *ordinary* type of chancriform sporotrichosis, except in the very early stages, the organisms are so sparse in the tissues as to be undiscoverable by histopathologic study; we would *expect* such *small* numbers or organisms to produce *little* or *no* complement fixing reactivity. It will be recalled also that in *primary cutaneous (chancriform) coccidioidomycosis* (the analog of the *ordinary* type of sporotrichosis), the titer of complement fixation is low or absent (as will be shown also in North American blastomycosis, see page 126) indicating a favorable prognosis. Conversely, *large* numbers of organisms can be seen *easily* in the tissues of infected animals, and probably of human beings dying of sporotrichosis. It is only in these *rare*, disseminated, *fatal* cases that the complement fixation should reveal a *high titer*, just as it is *very high* in the fatal disseminated type of coccidioidomycosis or blastomycosis.

It is also necessary to point out again that Norden autoclaved his sporotrichin, a procedure which experience

with *coccidioidin* would indicate should be avoided when preparing antigen to be used for *complement fixation testing,* since the ability to participate in the *complement fixation reaction is* removed from coccidioidin if it is subjected to prolonged heating. In place of heat, some other method of sterilization should be employed; perhaps the best suggestion might be that aqueous merthiolate 1-10,000 be employed, since it has been shown not to interfere with the reaction in coccidioidomycosis. Gonzalez Ochoa could not obtain complement fixation with his material, perhaps because some necessary protein-like component had been removed in "purifying" the polysaccharide by repeated precipitations with alcohol 96%. It appears likely that *the ability of an antigen to participate in the complement fixation reaction* depends upon its possessing some protein or protein-like elements, and that these may be easily destroyed or lost by many of the usual "purifying" or sterilization procedures. Before it is concluded that a complement fixation reaction cannot be developed into a reliable, consistent testing procedure in any disease, attempts should be made to produce antigens which have not been "denatured" in any manner which could degrade or injure any protein-like components which might be present. *Indeed, it would be advisable to investigate* all means by which any such "specific" protein elements could be increased in percentage or otherwise enhanced. This *should even be applied to tuberculosis.* (See also under blastomycin and its complement fixation reactivity, page 130)

A precipitin reaction has been reported to be routinely demonstrable in animal sporotrichosis, again conforming to recorded experience in the early phases of coccidioidomycosis Gonzalez Ochoa examined sera from nine known human cases of sporotrichosis and *found two*

strongly positive, two less strongly positive, three doubtfully positive and two negative by his precipitation test. One of the strongly positive sera came from the patient mentioned earlier who failed to react to the skin test. It was postulated that perhaps the "antibody" which was thus revealed as present in the circulating blood by the precipitin reaction was "saturating" the reactivity of the skin sufficiently to prevent its participating in the skin test. In coccidioidomycosis this thesis has been abandoned, since there is no evidence to show that the "antibodies" involved are similar or convertible one to the other, and attempts to "saturate" the skin in this manner have not been successful. Also, important is the fact that another of Gonzalez Ochoa's patients was skin test reactive in spite of high precipitin titer. If the precipitin test in sporotrichosis means the same as it does in coccidioidomycosis, it should be high early in the disease, and decline rather sharply after a few months (less than 7) without regard to the severity of the disease or whether the patient is destined to die or to recover. The duration of the disease in Gonzalez Ochoa's patients was not recorded.

It is evident that much additional data must be assembled before these tests may be adequately evaluated. One pitfall which must be avoided is that of anticipating the *same* results from *all* of these tests in a *given patient* at a *given time*, i.e., *all* tests *negative* or *all* tests *positive*. They do *not* so parallel each other in coccidioidomycosis; indeed it is evident that each test reveals the presence of a *different* antibody and that each is of *different* significance as far as the diagnosis of the disease as well as the prognosis of the patient is concerned. It seems likely that similar conditions will be found to prevail in sporotrichosis.

Therapy

The therapy of sporotrichosis is both easy and difficult to discuss, because although it is universally accepted that iodides act almost specifically and successfully, the mode of this action is still obscure. Potassium iodide is usually the drug selected, and the dosage should be soon raised as high as can be tolerated, up to 120 drops of the saturated aqueous solution daily, given in divided doses and taken in the usual fashion diluted with water or milk. In most cases the response is prompt and healing is completed within a few weeks. The medication should be continued for at least a month after all signs and symptoms have disappeared. If gastrointestinal intolerance develops, sodium iodide may be given intravenously in somewhat lower dosage, 1 to 2 gm. daily. Half strength Lugol's compound solution of iodine is useful as a wet dressing for open lesions.

Although the manner in which iodide exerts its beneficial action is still not clear, there are many clues. The concentration of iodide induced in the body tissues in general by the usual mode of administration probably cannot exceed 1-8,000, whereas it is known that *Sporotrichum schenckii* can be grown in culture in a medium containing up to 10% iodide (Gonzalez Ochoa). Using radioactive iodine (I-131) as a tracer, Shintani, and Wilson could not demonstrate any localized "uptake" by any of the cells of the host in sporotrichotic lesions in animals, nor any concentration in the areas of the lesions in general. Iodide therapy practically never fails in the ordinary chancriform type [illegible] f sporotricl [illegible] t is much less reliable in th [illegible] ted fo [illegible] vould seem to indicate that [illegible] degree [illegible] ologic resistance exerted by t [illegible] *ossessi* [illegible] criform type is of the great [illegible], and [illegible] id [illegible] simply

augment this resistance element in some small but vitally necessary factor. This would explain its failure in disseminated sporotrichosis where the resistance may be nil, or some inherent defect in the patients immunologic processes may be operative. (See also the status of stilbamidine in blastomycosis, page 141.)

There are insufficient data as yet to allow the value of iodide therapy to be examined in the light of the prognostic studies yielded by the interplay between the skin test and complement fixation titers, as has been recommended in coccidioidomycosis. It will be extremely interesting to watch for such reports.

Surgery is contraindicated since it apparently breaks the patient's wall of resistance and is often followed by spread of the infection. It is even recommended that *fluctuant accumulations of pus be treated by aspiration* rather than by incision and drainage for the same reason. For resolving granulation tissue moderately filtered x-radiation is helpful.

When progress is slow, vaccines prepared from the yeats-like phase of *S. schenckii* have been recommended as supplements to iodide therapy by several investigators. The status of such preparations is questionable, as in the other deep mycoses, particularly since vaccine-therapy has not apparently served to cure cases of the disseminated variety.

As with other deep mycoses, the usual antibiotics which are effective against bacteria should be withheld, unless they are *specifically* indicated because of concomitant obvious secondary infection with *susceptible* bacteria. *None* has as yet been shown to exert any inhibitory action against *Sporotrichum schenckii;* in fact, Gonzalez Ochoa and Soto-Pacheco have shown that *penicillin* acts as a *growth promoting factor*, and Campbell and Saslaw

attributed the same type of activity to *streptomycin.* Wide spectrum antibiotics may also deprive the body of some protein elements valuable in developing antibodies, by interfering with bacterial assistance in digestive processes. Some necessary vitamin B factors may be similarly inhibited.

Chapter 11

NORTH AMERICAN BLASTOMYCOSIS—HISTORY AND THE CLINICAL SYNDROMES

In 1894, Gilchrist presented to the American Dermatologic Association a preliminary report concerning a patient who possessed a skin disease unlike any which had been previously described, and which he attributed correctly to a microorganism appearing in the tissues as a budding yeast. Two years later he published a detailed study of this case, and in 1898 in collaboration with Stokes, he named the causative fungus *Blastomyces dermatitidis.* The disorder soon became known as blastomycosis, or Gilchrist's disease. Numerous similar cases were reported during the next few decades, including some in which the disorder involved internal organs as well as the skin, but it became apparent there was considerable confusion with the other deep fungous diseases in which the organisms in the tissues can appear as spherules, namely cryptococcosis (torulosis), coccidioidomycosis, and South American blastomycosis This confusion was not resolved until the period around 1930, when methods of differentiating among these diseases were clarified by Almeida and by Benham.

During the half-century which followed the original discovery, it became firmly established that North American blastomycosis exists in two forms, the clinical pictures of which are markedly different. In one, the skin alone

is apparently involved in a chronic, ulcerative, granulomatous process, slowly progressing at the periphery and healing by cicatrization in the central portions, and continuing for many years without usually affecting the general health of the patient. In the other, the lungs are the primary focus, from which the disease as a rule soon becomes widely disseminated, usually causing death. Rarely was a combination of these two forms reported or even a transition from one to the other.

It became universally accepted that this sharp differentiation was attributable entirely to the manner in which the infecting fungi had entered the body, inoculation of the organisms directly into the skin causing the chronic cutaneous form, while their inhalation into the lungs resulted in the disseminated visceral type. By 1951, however, Schwarz and Baum after extensively studying blastomycosis from the pathologist's viewpoint, had discovered that painstaking investigation of cases of the chronic cutaneous type usually revealed evidence of concomitant or preceding pulmonary involvement; they were so impressed with the *regularity* of this finding in so large a *percentage* of such cases that they became convinced that it probably *exists* in all, even though too minor or evanescent to be *detectable* in some. They concluded that the chronic, cutaneous type was therefore simply another manifestation of the way in which dissemination from a pulmonary focus could evolve, contrasting sharply with the widespread visceral involvement previously alone considered typical.

In support of this view they pointed out that there were three instances in which *Blastomyces dermatitidis* was *known* to have been inoculated directly into the skin of previously uninfected individuals, and in no case had the ordinary chronic, cutaneous form of the disease re-

sulted. On the contrary these patients had all exhibited a chancriform picture, closely resembling the usual form of sporotrichosis (see page 79). The following year, I was privileged, among others, to observe the case of a mortuary attendant who became infected with *B. dermatitidis* by means of a puncture wound sustained into a finger while preparing the body of a person dead of the disseminated form of blastomycosis. The clinical picture which evolved was typically *chancriform* and in no way resembled the *ordinary chronic cutaneous type*. (This spectacular event appeared entirely convincing to me, because I had been by that time already "conditioned" by closely observing for 5 years the course of a patient intracutaneously inoculated in exactly the same manner with *Coccidioides immitis*, and in whom the identical *chancriform* syndrome developed.) Subsequently Wilson, Cawley, Weidman and Gilmer studied all four of these cases and reported them in detail, considering the evidence sufficiently conclusive to establish this type as an *entity*, called *"primary cutaneous blastomycosis."* Although this view has not as yet been universally accepted, it will be adhered to in the classification used here.

Primary Cutaneous Blastomycosis

Although extremely rare and accordingly warranting no such priority, this type will be first disposed of here, because the other more common forms merge into one another in such a manner that continuity in their discussion is desirable.

In the four known cases, primarily inoculation of *B dermatitidis* into the skin of human beings resulted in a papule appearing at the inoculation site in a week or so, followed in about two weeks by lymphangitis and lymphadenopathy remaining localized to the affected limb.

In two cases several tender nodules developed along the course of the lymphangitic vessels, resembling closely the usual picture of sporotrichosis. Although the evidence is not conclusive, it appears probable that blastomycosis acquired in this manner has a tendency to be much milder than in the other forms, and even to heal completely and spontaneously in most instances. The primary lesion was excised in three cases, and the lymph-nodes in the fourth, but in no case were *both* of these infected areas extirpated Nevertheless all patients became entirely healed within four months, without developing either the chronic localized cutaneous form of the disease or sustaining dissemination. Subsequently all of these patients showed no recurrence, one during 44, one 29, one 6 and one 4 years, respectively.

Histopathologic preparations reveal the primary lesion to consist of a dense infiltrate of leucocytes predominantly of the polymorphonuclear type, within which are to be seen the singly-budding cells of *B. dermatitidis.* In the early phases of the infection these organisms are numerous, later they are found to have decreased markedly in numbers. The lymphangitic streak reveals an inflammatory infiltrate containing a multiplicity of cell types and numerous blastomycetes. The lymph nodes reveal *foci of acute granulomatous lymphadenitis* in which are giant cells occasionally containing the fungous organisms.

Primary Pulmonary Blastomycosis

Although much evidence indicates that the lungs are frequently (if not indeed almost invariably) the portal of entry for blastomycosis, the resulting disease is so insidious in its development that little is known of the primary phases. The source in nature from which the organisms are acquired has not as yet been discovered. In

spite of careful search directed along the lines so successful in the case of the other deep mycotic fungi. It is virtually certain however that the spores must be inhaled in the manner which has become the established fact in coccidioidomycosis and probably in histoplasmosis.

At first the infection apparently closely resembles an ordinary subacute respiratory infection, presenting itself as a nonproductive cough accompanied by moderate fever, chest pain and perhaps dyspnea. These symptoms gradually increase in severity, bloody and purulent sputum appears, and weakness, anorexia and loss of weight begin to be prominent. The fever becomes more pronounced and night sweats often occur. Pleurisy is occasionally observed. Erythema nodosum has been recently reported, reminiscent of its frequent accompaniment with coccidioidomycosis in the same stage

Little is known of pulmonary blastomycosis in any mild form, such as is recognized in coccidioidomycosis. When severe, it is difficult to differentiate pulmonary blastomycosis from massive tuberculosis or lung abscesses by physical examination, unless the infection erodes through the chest wall to form discharging sinuses in the skin X-ray examination of the chest may reveal extensive enlargement of the mediastinal nodes, but this is often hidden by massive densities projecting irregularly from that area, in a manner strongly suggesting bronchogenic carcinoma. There may be only unilateral involvement at first, but later the other lung usually becomes infected. Cavity formation is not common, and such lesions are usually small and irregular in outline Occasionally there is evidence of massive miliary spreading Sometimes the patient slowly recovers; sometimes death occurs while the disease is still confined to the lungs; more frequently, however, dissemination to extrapulmonary areas occurs.

Pathologically, pulmonary blastomycosis may grossly resemble carcinoma or tuberculosis. Nodules of all sizes some of which are caseous, abscesses, and occasionally cavitation are seen. Pleural thickening is common, and frequently the disease extends beyond the pleura to involve the ribs, and sometimes even to penetrate the chest wall and discharge externally. Involvement of the lungs is almost always extensive in patients dying of blastomycosis, and the organisms are usually found to be present in enormous numbers accompanied by a comparatively slight degree of cellular inflammatory reaction.

Disseminated Blastomycosis

Disseminated blastomycosis occurs by means of hematogenous spread of the fungi from the lungs to other parts of the body. The skin is involved in a large percentage of cases, and the bones in perhaps two-thirds, most commonly the ribs and vertebrae. Bony lesions seen in x-rays reveal both destructive and proliferative processes, resembling those produced by tuberculosis more than the cyst-like lesions of coccidioidomycosis, but less proliferative than actinomycosis Compression of the spinal cord may result from the collapse of vertebral bodies.

Involvement of the viscera is common, especially the liver, spleen, kidneys and prostate. In about one third of the cases central nervous system lesions occur, and meningitis or brain abscesses are not rare. In striking contrast to South American blastomycosis, the intestinal trac is usually spared.

Anemia of the hypochromic type is typically present and there is usually a leucocytosis, predominantly poly morphonuclear. The sedimentation rate is heightened

Pathologic examination in *disseminated blastomycosi* usually reveals involvement of many organs, and the le

sions are seen to contain ever more numerous organisms in proportion to the degree to which the disease has progressed and the efforts of the tissues to combat the infection have become lessened. In the earlier stages there is a purulent character to the lesions; later, tubercles, necrotic zones and abscesses appear. Bony lesions frequently result in subcutaneous abscesses or sinuses draining through the skin. There may be focal lesions in muscles. Pericarditis with effusion has been reported.

In autopsy material the organisms may be present in such large numbers as to appear almost as though in pure culture, the body of the host having long since ceased to offer much immunologic or cellular resistance to their multiplication. As will be discussed in the next paragraphs, this picture is in striking contrast to that seen in the chronic cutaneous form.

Chronic Localized Cutaneous Blastomycosis

In this, its most common form, North American blastomycosis is first observed as a cutaneous lesion and remains apparently limited to the skin throughout a chronic course extending over many years. As has already been pointed out it was until recently assumed that this type of infection was caused by direct inoculation of the organisms at the point where the initial lesion appeared. Since the realization that in those four cases in which cutaneous inoculation was *known* to have occurred, an entirely different *chancriform* syndrome developed, it has become necessary to explain this chronic cutaneous form as simply a *special* type of the *disseminated* disease.

Regardless of arguments as to its mode of origin, localized cutaneous blastomycosis begins either as an isolated papular lesion in or just below the skin, or as a subcutaneous nodule developing into an abscess and eventu-

ally rupturing to form an ulcer. The lesion soon develops a verrucous appearance, studded with tiny pustules, the whole being raised above the level of the surrounding skin a few millimeters. There is slow peripheral extension, until the lesion is a few centimeters in diameter, at which time the central area begins to subside, finally healing completely by the formation of a soft atrophic non-contractile scar. Other lesions may appear, either originating as did the first from the primary pulmonary focus, or by direct transplantation of the organisms from the skin lesions to a new area of the skin, and by irregular peripheral growth and coalescence, gyrate, arciform and serpiginous forms are produced. Over many years this process may eventually cover large areas of the body surface, perhaps as much as a sixth of its extent, but there is a strong tendency to remain *roughly* contiguous to its point of first appearance.

Typically at the borders of these lesions there is a characteristic verrucous ridge a few millimeters in height, with many pustules and exudative crusts on its crest, the whole resting on a tumid, violaceous base which terminates abruptly at the edge of the normal surrounding skin centrifugally, and equally precipitously in scarred atrophy at the center. This entire process has been appropriately likened to a fire in a field of grass or grain; islands of skin are often left uninvolved or islands of activity may remain long after they have been separated from the main mass of the infection. In contrast to syphilis, which never returns to areas it has once abandoned, there are instances of recurrences in areas already scarred by blastomycosis.

In chronic localized cutaneous blastomycosis the pathologic picture *differs markedly* from those forms previously described. *Here* there is acanthosis, developed to a more marked degree than in *any other known disease,*

assuming to the *highest degree* the condition called pseudoepitheliomatous hyperplasia, frequently mistaken for squamous cell carcinoma. Numerous micro abscesses are seen, many of which are entirely contained within the thickened epidermis, and which contain pus composed largely of polymorphonuclear leucocytes and some lymphocytes. Within these microabscesses an occasional fungus cell can be seen; in fact this is the *most adventitious* location for these organisms to be sought, and yet *even here* they are so sparsely distributed that the experienced histopathologist will request that many serially-cut sections be furnished to him because he may have to search through a dozen or more before being rewarded. Below the extremely irregular boundary of the epidermis, there is a band of dense infiltrate consisting of leucocytes among which polymorpho-nuclear cells predominate. Giant cells are often seen, some of which contain fungous elements. Within all of this active mass there is practically no connective tissue stroma, but many dilated blood vessels are present. Fungous cells can be discerned here only rarely. In sections taken from areas in which the disease is subsiding, fibrosis is present, but it is usually not firm nor dense.

The diagnosis of North American blastomycosis can be made by the discovery of the typical organisms in direct microscopic examinations of exudates, or in histopathologic preparations of tissues. The periodic acid-Schiff stain (Hotchkiss-McManus, popularized by Kligman and Mescon) is a valuable adjunct, since it stains fungus cells differentially. *Blastomyces dermatitidis* can usually be recovered in culture, but the technique is not easy and often requires specialized media and incubation. Diagnosis by serologic reactions and skin tests is also possible, as will be discussed later.

Chapter 12

NORTH AMERICAN BLASTOMYCOSIS–THE SIGNIFICANCE OF LONG PERSISTING FOCI OF CUTANEOUS INFECTION

IN THE PRECEDING chapter some recent evidence was presented indicating that there is need for revision of some of the previously held concepts of the pathogenesis of North American blastomycosis, especially with regard to the clinical picture which results from the intracutaneous inoculation of this fungus into the skin of previously uninfected person. It had always been accepted that when acquired in this manner, blastomycosis assumed the chronic, localized cutaneous form and endured for years without endangering the life of the patient. Similarly, coccidioidomycosis had been accepted as behaving in the same way until the patient of Wilson, C. E. Smith and Plunkett, who was known to have been infected intracutaneously, subsequently followed a chancriform course instead. In an entirely analogous fashion, the work of Schwarz and Baum, followed shortl by the study made by Wilson, Caw man an appeared to establish prima s blasto so as a chan criform syndrom le it n o accept the view that the usu utane sults by dis on of th the an i ternal us. p pul

When these radical new ideas were tested in application to the other phases of blastomycosis, it became apparent that a theory could be evolved which would serve much better than any previously available to explain certain discrepancies in our understanding of its pathogenesis, as well as some apparent inconsistencies encountered in studying its immunologic behavior. These factors will now be presented in some detail.

First of all, it must be admitted that there are many points concerning which additional knowledge about primary cutaneous blastomycosis is to be desired. However, it is evident at once that the syndrome which followed the intracutaneous inoculation of *B. dermatitidis* was identical in most respects in the *four known cases*, and that it differed remarkably from the chronic cutaneous form of the disease which previous opinion would have led one to anticipate. It must be considered significant that these four patients, who are the only ones actually known to have been infected percutaneously with blastomycosis from *a proved* source of the organisms are also the only ones who developed a primary chancriform lesion at the known inoculation site, accompanied by lymphangitis and lymphadenopathy limited to the involved extremity. Furthermore, in *none* of these four patients did the disease evolve into the syndrome which has always been the prototype of the "cutaneous" form of blastomycosis, i.e., a slowly enlarging cutaneous plaque with a sharply delineated, elevated, verrucous, pustular, violaceous border and a clearing cicatricial center, *not* accompanied by lymphadenopathy.

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When these radical new ideas were tested in application to the other phases of blastomycosis, it became apparent that a theory could be evolved which would serve much better than any previously available to explain certain discrepancies in our understanding of its pathogenesis, as well as some apparent inconsistencies encountered in studying its immunologic behavior. These factors will now be presented in some detail.

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That the inoculation of B. dermatitidis into the skin produces a chancriform syndrome should not be surprising, in the light of our knowledge that other microorganisms which cause infectious granulomatous diseases operate

similarly under similar circumstances. With but minor variations the same picture is observed in the primary cutaneous expression of tuberculosis, syphilis, yaws, American leishmaniasis, sporotrichosis, and coccidioidomycosis (see page *91*). The nodules along the lymph channels observed in two of these cases of blastomycosis simulated those so characteristic of sporotrichosis and those encountered in the case of coccidioidomycosis reported by Wilson, Smith, and Plunkett.

Additional support for the above thesis is to be had by observing how well it is able to clarify some hitherto puzzling clinical and immunologic aspects of blastomycosis. It now appears likely that, except when inoculated intracutaneously, blastomycosis follows in general the pattern *which has become so well understood* in coccidioidomycosis, which is represented in skeletal form by the following sequence of events: (1) many persons inhale the fungi; (2) a large proportion acquire the disease in the lungs; (3) some die, but many resist the disease so efficiently that it is kept localized in the lungs and remains entirely asymptomatic and subclinical until it eventuates *in complete recovery;* (4) *some* persons do *not quite so* efficiently resist the infection, and these sustain hematogenous dissemination of the fungi to other organs, one of the commonest of which is the skin; (5) some of these persons die; (6) some continue to resist the disease immunologically and finally eliminate it entirely; (7) others succeed in clearing the infection completely from all internal organs, but cannot quite *eliminate it from the skin,* which may then continue to be involved for years in a chronic cicatrizing form. Deferring for the moment the immunologic and allergic implications, some clinical observations are in order, since at least a *few* cases of *blastomycosis* represent

ing any desired stage of the above sequence of events have been observed.

Fatal pulmonary blastomycosis without dissemination beyond the lungs has not been frequently recorded but it is extremely likely that it exists more often, many such cases undoubtedly having been not recognized as caused by this organism, but simply classed in spite of the lack of proof as tuberculosis, pneumonia or the like. Routine use of the complement fixation test with blastomycin may sometime serve to reveal the true status of the incidence of this type of infection, since the titer should be significantly high, unless death occurs too rapidly.

That there are persons who acquire pulmonary blastomycosis, but maintain it entirely in a subclinical phase or exhibit only mild symptoms while they successfully develop complete immunologic resistance, is not well established as yet It will be recalled that this is the *rule* in coccidioidomycosis, over 60% remaining entirely asymptomatic during the entire process. This is revealed by the high percentage of persons who, after a few years of residence in the known endemic areas, develop the ability to react specifically to the skin test with coccidioidin. Blastomycin has not as yet been utilized in surveys covering as large numbers of "normal" persons as has coccidioidin, but it *has* been used sufficiently to warrant the conclusion that even in the most "endemic areas" for blastomycosis there is no *large percentage* of the population who react to it, revealing thereby a previous "silent" infection Several possible reasons for this variation come immediately to mind; the organisms are almost certainly not as profusely distributed in the environment as in the case of Coccidioides (indeed the reservoir in nature for *B. dermatitidis* has not as yet been discovered), the organisms are therefore probably not inhaled by any *large numbers* of

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people in sufficient quantities to transmit the infection; or they are relatively non-virulent as compared to *Coccidioides*. Perhaps all of these factors are operative. It seems certain also that the human body does not acquire effective immunologic resistance to blastomycosis as easily as it does to coccidioidomycosis, so that probably a much higher percentage of those persons woh *do* become infected, develop it in a *serious* form.

It is quite probable however that there are *some* such "silent" cases of blastomycosis. In this regard it must be considered extremely significant that recently Smith, Harris, Conant and Smith, while investigating a small, but highly interesting and instructive epidemic in North Carolina, found in that vicinity several "normal" persons who reacted positively to the intracutaneous injection of blastomycin, and who almost certainly therefore must be presumed to fall into this category even though no such clinically evident illness was recalled. So may some of the so-called "false positive" reactors to the skin test reported from time to time by other observers. Further developments in this field are to be anticipated.

There have been many instances of recognized pulmonary blastomycosis which have been accompanied by lesions in other organs or in the skin indicating dissemination; sometimes indeed, the infection has been recognized while *still localized* in the lungs, and the process of dissemination *subsequently* closely observed from its *very beginning*. I know of no cases, however, in which this sequence has been clearly proved to have been reversed, that is for skin lesions to be present while the lungs were demonstrably clear, only to have them become involved later. In other words, dissemination seems to occur in one direction only, from the lungs to the skin and never by the reversed route. (That this *might* occur following

primary cutaneous inoculation cannot be denied, but it has not as yet been demonstrated, the four known cases of this type having failed to spread in this manner.) Moreover, when pulmonary blastomycosis is present in an *active stage accompanied* by skin lesions, the latter usually can be shown to have first appeared but a *short* time previously, usually less than a year. Conversely, in those cases of the *chronic* cutaneous type in which the history indicates that the skin infection has been present for *many years*, the lungs are usually *not* found to be actively involved. It is logical therefore to conclude that in these cases the lungs (which, according to the recent concept, were always the *original primary* focus) have long ago *healed*, and are thereafter no longer susceptible to reinfection "backwards" from the skin. Carrying this inference still further, it appears that a patient will either die early in the course of disseminated blastomycosis, or will resist it immunologically to a sufficient degree as to clear it entirely from the *lungs* (leaving them thereafter immune to reinfection), but not always completely from the *skin*, which may continue to be involved for many years in the chronic, cutaneous form. That this chronic form, so well known for sixty years and attributed formerly to direct cutaneous inoculation, is instead actually the residual of an old dissemination from a primary pulmonary focus is a recent concept not as yet generally accepted, but this author believes that there is ample evidence to prove that it is correct. Most important of all is the recently demonstrated fact that this type of disease did not develop in *any* of the four cases *known* to have acquired the infection by skin inoculation. The allergic and immunologic reactions, which will be discussed soon, also point in this direction.

How, then, can we account for those cases of chronic cutaneous blastomycosis in which there is no recollection of any preceding pulmonary disorder which could have been the primary focus? It is likely that this follows the line established by coccidioidomycosis, and simply means that pulmonary blastomycosis may be present but remain entirely asymptomatic, or may be so mild as to be classed as a "cold" or "influenza" and *yet* be capable of disseminating in *susceptible* individuals, after which the lungs may become clear, still without that involvement having been realized. This also strengthens the theory that pulmonary blastomycosis may have existed in a considerable number of persons and become completely healed without ever occasioning any concern, furnishing thereby positive reactors to blastomycin among presumably "normal" individuals. It will be asked why the infection does not spread from the skin backwards to the lungs once they have become cleared. This cannot be explained as due to any lack of virulence of the infection, because the skin disorder cannot be said to be inactive; on the contrary, it frequently extends *locally* to an astonishing degree and lasts for many years, eventually involving perhaps as much as a *sixth of the body surface*. Cannot the infection thus become sufficiently virulent again to "disseminate" once more, this time inwards to the lungs or the viscera? Perhaps this *does* in fact occur occasionally, by eventually debilitating the patient, but I doubt that it is frequent. In fact it is difficult to find cases in which this even seems *likely* to have occurred. It is more logical to conclude that the lungs became cleared *originally* only because they developed an effective degree of *immunologic resistance*, and that this resistance then persists for years or for life *in that area.*

It may seem odd to claim that the *lungs* of an individual can thus possess the ability to resist the disease completely by immunologic means; while the *skin* obviously in many cases does not, since it may continue to be involved for years. However there is an explanation which seems to fit and which is apparently supported also by certain allergic and immunologic phenomena soon to be discussed. From a clinical and pathologic standpoint it is evident that, while the skin frequently fails to develop *complete resistance* (that is, sufficient to achieve a spontaneous *cure*), there are several reasons to believe that it nevertheless *resists* the infection *intensely*. First, it must be noted that many cases have been observed in which the skin has been involved continuously and extensively for many years without the general health of the patient being affected. With an infection *potentially* as virulent as blastomycosis, it can only be concluded that it is being resisted *very well* by the other parts of the body in these instances Second, the skin succeeds in healing itself completely in the central portions of the usual type of chronic cutaneous blastomycosis, again a manifestation of high resistance.* Third, anyone who has succeeded in establishing the diagnosis in this type of blastomycosis by histopathologic study cannot but be deeply impressed with the difficulty with which the causative fungi are to be discovered, they are usually present in such infinitely small numbers that several sections must be examined before one organism can be seen In contrast, the *cellular* reaction of the host which these sparse organisms engender in the skin is tremendous, a dense infiltrate consisting of thousands of lymphocytes and plasma cells for every

*Not as complete however as is exhibited in syphilis for in blastomycosis such healed areas may become infected again by direct inoculation

fungus cell, contained beneath an epidermis so highly stimulated that it closely resembles a carcinoma (pseudo-epitheliomatous hyperplasia). This certainly bespeaks a high degree of *resistance.*

Yet, although the *resistance* in the skin is of high degree (indicating that it also participates in the immunologic reaction which succeeded previously in clearing the lungs completely) it frequently fails to achieve complete success. There must be some relatively small defect in the mechanism of resistance which falls just a little short of perfection. The nature of this deficiency cannot be defined at present, but it seems to offer a most promising field for research. *Two other facets of this phenomenon* will soon be pointed out.

Chapter 13

NORTH AMERICAN BLASTOMYCOSIS—IMMUNOLOGIC ASPECTS

As early as 1914, Boughton and Stober had utilized an extract from cultures of *Blastomyces dermatitidis* as a skin testing material, both by the scratch method and by intracutaneous injection. Their patient showed a "positive skin test to the latter route." It will be seen to be highly significant that they also stated that their patient was "recovering from the infection at the time the test was made."

By 1941, Martin had become convinced that two immulologic testing procedures were of value in ascertaining the prognosis in cases of blastomycosis. He observed that patients dying of the infection usually reacted only minimally, or not at all, to the intracutaneous injection of a substance (blastomycin) extracted from cultures of *B. dermatitidis*, while their sera were able, in conjunction with this antigen, to fix complement in a high dilution (up to 1-32). Conversely, patients having only a small, chronic, cutaneous focus which persisted for many years, usually reacted strongly to the intracutaneous test, while their complement fixation reaction was either negative or only weakly positive At about the same time, students of coccidioidomycosis, especially the groups working with C. E. Smith and Kessel, were independently making similar observations on patients with that disease. Subsequently, after thousands of tests on hundreds of patients

with *coccidioidomycosis*, it has been established that for purposes of prognosis the clinician may use the reaction to the intracutaneous test (roughly quantitated by using the degree of response to several dilutions) as a measure of the extent of the immunologic resistance which the patient is mobilizing against the disease, while the reaction to the complement fixation test (also quantitated by dilutions) serves to measure the extent and severity of the disease, or "the amount of tissue involved," or in a later view "the number of the causative organisms actively engaged in producing the disease at the time of the test." It must not be implied, however, that any of the *antibodies* revealed by these tests have been shown to be the *actual means* by which *immunity is conferred upon the individual*, but rather that there is a high degree of *parallelism* useful to the *clinician* for ascertaining the *prognosis*. *These facts have become clearly evident earlier in coccidioidomycosis*, principally because of the opportunity for study afforded by the large number of persons who manifest *the infection in its extremely benign form, a stage not* as yet demonstrated conclusively in blastomycosis. However, the same views seems likely *to prove valid in the latter disease also, and are given credence by many observers.* In recent years there is considerable evidence indicating that histoplasmosis *follows the same pattern*

It is noteworthy that in the only four known cases proved primary cutaneous blastomycosis, the intradermal tests (which were performed *only in* the two later cases), became positive in a significantly *high dilution* rather early, indicating the development of *good immunologic resistance*, while the complement fixation titer was persistently zero in one case *and very low in another*, indicating but a small *extent* of *infection* The acceptance of these indications of a good prognosis was borne out by the

clinical course which the disease followed in these patients, all four of whom recovered completely without any therapy which could be considered in the least specific, and with only partial surgical extirpation in any of the cases.

In many instances of pulmonary blastomycosis the complement fixation titer has been found to be high (high, that is, for blastomycosis but *not* when compared with titers in coccidioidomycosis) while the skin has reacted minimally or not at all. The interpretation of these phenomena as indicating a poor prognosis is supported by the high rate of fatality actually observed in this type of blastomycosis. *Some* of these patients have also simultaneously had disseminated lesions in other viscera or in the skin, and in these persons the complement fixation titers have usually been correspondingly higher. *Some* others have shown a reversal of these tests, however, indicating a good prognosis, and of course some of these have recovered, bearing out this prediction

The reactions to blastomycin exhibited by patients who have possessed the *chronic cutaneous* form of blastomycosis without concomitant pulmonary lesions being evident have, until recently, been considered "erratic," and difficult to interpret However the recent concept that this form is derived by dissemination from a pulmonary primary focus is capable of explaining most of these discrepancies. For example, some cases have shown a high complement fixation titer and a low skin test reactivity: and some of these have been shown to possess the infection still in the active form in the lungs or other viscera as well, thereby accounting for the large amount of heavily involved tissues indicated by such a high titer. Thus, a titer higher than that which *should be expected* from the skin lesions which are *visible* should therefore be taken as

with *coccidioidomycosis,* it has been established that for purposes of prognosis the clinician may use the reaction to the intracutaneous test (roughly quantitated by using the degree of response to several dilutions) as a measure of the extent of the immunologic resistance which the *patient is mobilizing against the disease, while the reaction* to the complement fixation test (also quantitated by dilutions) serves to measure the extent and severity of the disease, or "the amount of tissue involved," or in a later view "the number of the causative organisms actively engaged in producing the disease at the time of the test." It must not be implied, however, that any of the *antibodies* revealed by these tests have been shown to be the *actual means* by which immunity is conferred upon the individual, but rather that there is a high degree of *parallelism* useful to the *clinician* for ascertaining the *prognosis.* These facts have become clearly evident earlier in coccidioidomycosis, principally because of the opportunity for study afforded by the large number of persons who manifest the infection in its extremely benign form, a stage not *as yet demonstrated conclusively in blastomycosis.* However, the same views seems likely to prove valid in the latter disease also, and are given credence by many observers In recent years there is considerable evidence indicating *that histoplasmosis follows the same pattern.*

It is noteworthy that in the only four known cases proved primary cutaneous blastomycosis, the intradermal tests (which were performed only in the two later cases), became positive in a significantly *high dilution* rather early, indicating the development of *good immunologic resistance,* while the complement fixation titer was persistently zero in one case and very low in another, indicating but a small *extent* of *infection.* The acceptance of these indications of a good prognosis was borne out by the

clinical course which the disease followed in these patients, all four of whom recovered completely without any therapy which could be considered in the least specific, and with only partial surgical extirpation in any of the cases.

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strong evidence that there are *other* lesions in the *viscera* or *lungs* as well, perhaps subsequently discoverable by careful examination, but sometimes existing in "silent" areas.

By contrast, many cases of extensive chronic cutaneous blastomycosis have revealed low complement fixation titers and a high degree of skin test reactivity. This has frequently appeared to be paradoxical for two reasons: first, such a large area of involved tissue would be ordinarily expected to produce high complement fixation activity, and second, the duration and extension of the disease during many years belied the "good prognosis" indicated by the high skin test reactivity. These results are not at all inconsistent however, if (in accordance with the latest concept) complement fixation titer indicates the "number of organisms actively engaged in producing the disease at that time"; for in this type of the disease the organisms are indeed few in total numbers. That the high skin test reactivity actually indicates high resistance is also consistent, *if the view advocated above is accepted, that is that the skin in this type of disease is actually exerting a high degree of resistance, even though it does not succeed* in achieving a *complete* cure.

It is necessary to consider the nature of this material called "blastomycin" with the assistance of which these tests are performed. Recent work by Martin has demonstrated that probably both polysaccharide and protein elements are produced by the yeast phase of *Blastomyces dermatitidis* in culture (i.e., that grown at 37° C.). By simply washing these budding cells with saline a solution is obtained which fails to produce a precipitate with trichloracetic acid or to react to the biuret test indicating the absence of more than tiny amounts of protein; *yet it yields a strongly positive Molisch reaction indicating polysaccharide (carbohydrate)* This material serves well for the

performance of the intracutaneous test in human beings possessing blastomycosis, but does not fix complement with their sera. Martin does not state whether or not this material is entirely nitrogen free, so that it may still contain some protein-like haptene groups, although it is essentially protein-free.

In his earliest experiments with complement fixation in blastomycosis, Martin utilized a saline suspension of living *Blastomyces* yeast phase cells as the antigen and obtained satisfactory reactions. Attempts to make similar potent antigens which were soluble and stable by freezing and thawing or by mechanical grinding were unsuccessful. Recently he has made a soluble antigen satisfactory for complement fixation reactions in human sera by liberating the active principle from the yeast cells by treatment with the sonic oscillator for 15 minutes (In fact, this material was made from cells remaining after the removal of the polysaccharide component described in the preceding paragraph) This solution gave strong reactions to tests for both carbohydrate (polysaccharide) and protein.

It will be recalled (see page 45) that coccidioidin from which practically all nitrogen has been removed (therefore protein free) still serves beautifully for the performance of the skin test, even as Martin's purified polysaccharide "blastomycin" serves in blastomycosis Coccidioidin as *ordinarily* made serves very well however and yields such a high degree of specificity that purification by removal of its small amount of nitrogen is not necessary. However, there is evidently a great deal more nitrogen in the form of protein in the cells of *Blastomyces* than in *Coccidioides*, and such *protein* may well be responsible for some of the "non specificity" exhibited by some of the "blastomycins" used in the past. It would seem advisable to use exclusively such a purified polysac-

charide product as that of Martin for *skin* testing in blastomycosis in future investigations.

Conversely, since apparently a protein-like component is necessary if an antigen is able to enter into the *complement fixation* reaction, Martin's *protein* component would seem to be preferable for this purpose over any less purified mixture, although it still contains polysaccharide elements as well (so does coccidioidin need nitrogenous structures for complement fixation activity although also containing polysaccharide).

Martin has also observed that a precipitin reaction can be made to occur with blastomycosis sera, but has not studied this procedure sufficiently as yet to define its usefulness or significance. In addition he is studying the phenomenon of hemagglutination (agglutination of sheep's erythrocytes previously sensitized by blastomycin when mixed with serum from patients possessing blastomycosis) in the manner suggested by the work of Middlebrook and Du Bos in tuberculosis. This reaction needs much more investigation before it can be defined.

Such studies as these leading to the preparation of testing materials of greater reliability, specificity and stability over long periods of time are extremely valuable and when carried out with regard to the specific antigens for other fungous infections should do much to clarify our understanding. As far as can be seen at this time, there are great similarities in the nature of the materials and the significance of their reactions in all fungous diseases thus far investigated.

Chapter 14

NORTH AMERICAN BLASTOMYCOSIS—APPROACHES TO THERAPY

NORTH AMERICAN blastomycosis has always been considered by most physicians to be a serious disease, proceeding if untreated almost *uniformly* either to the death of the patient or to an extensive involvement of the cutaneous surface of the body in a horrible, purulent, crusted, granulomatous involvement to which death might be preferred. With such devastating examples appearing all too frequently, it has become almost the universal custom to *ignore entirely* the possibility of the occurrence of spontaneous recovery, and to attribute *all* of the improvement which *any* patient has obtained while under *any form* of medical supervision to the *treatment* methods being employed. The result was that some therapeutic items gained sufficient reputation to cause them subsequently to be employed in *most cases,* even though their most enthusiastic advocates could not avoid admitting that the response was all too often *not in the least satisfactory.* Blastomycosis thus became established as a disorder highly capricious in its reactions to treatment.

However, there is *now* sufficient evidence to believe that spontaneous recovery by means of the body's own immunologic processes *occurs not rarely,* and possibly frequently For example, the only four instances of *proved intracutaneous inoculation* of *B. dermatitidis* resulted in

complete recovery in spite of incomplete surgical intervention and the absence of *any* consistent type of therapy. The recent discovery by Smith, Harris, Conant and Smith that some "normal" individuals living in the vicinity of an "epidemic" of blastomycosis were able to react positively to intracutaneous testing with blastomycin makes it appear likely that they had been previously infected and recovered without even enough sickness to be remembered. The recent concept that chronic cutaneous blastomycosis is always the residual of a disseminated process originating in the lungs entails the acceptance of the fact that such pulmonary involvement *can* become healed completely by spontaneous means before causing sufficient illness to be memorable, leaving only roentgen shadows as evidence. There must also be many cases of pulmonary blastomycosis which do not disseminate, and which clear completely by spontaneous means without ever being correctly diagnosed. In the preceding chapter it has also been pointed out that in the chronic cutaneous form, the skin fights diligently even though it seldom succeeds completely in overcoming the disease.

Thus it is logical to conclude that at least *some* of the "capriciousness" in the response of blastomycosis to various types of therapy can be explained by variations in the degree to which the *patient* fights the disease by his own *immunologic* mechanisms. Accordingly it is of paramount importance to be able to determine accurately for each individual patient what the result would be if *no therapy* at all were administered. Not until a basis such as this can be established can *any* type of therapy be *accurately* evaluated

Fortunately this is not a remote hope, for the information revealed by the use of blastomycin in the intracutaneous test and in the determination of the complement

fixation titer in the patient's serum are of great prognostic value. Patients exhibiting a *low* reactivity to the *skin test* and a *high titer* by the *complement fixation reaction* are in serious danger of death. The outlook is entirely reversed in those persons who react well to the *skin test* while the serum *fails to fix complement* with appropriate antigen If all physicians who care for patients afflicted with blastomycosis could be convinced of the value of these tests sufficiently to employ them at intervals of every few weeks during the course of the disease, a very few years of such observations would probably clarify many of its "capricious" responses to therapy. *All* drugs and methods, old as well as new, should be thus accurately evaluated lest they be credited with a beneficial effect which would have occurred equally well without them, and later administered to other patients because of such unjustified faith.

Potassium Iodide

Potassium iodide was used in some of the earliest cases, with apparent benefit in some and complete failure in others. Nevertheless, it became customary to employ it in *all* cases, probably largely for want of something better, but undoubtedly partly because of its dramatically successful effect upon sporotrichosis It has usually been administered orally to the point of the patient's tolerance, up to 150 drops of the saturated aqueous solution in divided doses daily over many months With the recent advent of stilbamadine, KI has been much less frequently employed, but it is evident that it cannot be thus *entirely* replaced since there are *also failures with* stilbamidine David T Smith and his collaborators have warned about the danger of the use of potassium iodide in persons who are *highly* reactive to the *skin test,* since some such pa-

tients have become subsequently much worse, apparently because of a deleterious hyper-allergic reaction to the products of the fungus organisms killed by the iodide. They recommend inducing a significant degree of desensitization by administering increasing doses of blastomycin intracutaneously before iodide therapy is begun. This has always been difficult for this author to understand, since, according to the concept to which he adheres, it is this very ability to react to the skin test with blastomycin which indicates that the patient possesses a significant degree of immunologic resistance, and any reduction of this by "desensitization" might *retard* the patient's progress rather than assist him. This subject impinges so directly upon that of "vaccinotherapy" which will be discussed next that it will not be treated further under this heading.

Vaccinotherapy

Vaccinotherapy is advocated principally by the group of investigators (D. T. Smith, Martin, Conant, Callaway and Baker), centered about Duke University, who have studied North American blastomycosis most intensively. This author admits gratefully that much of his present understanding of this disease was learned from the writings and lectures of these men and it is with considerable reluctance that any views which conflict with their opinions are set forth. However, there are some discrepancies which I have not been able to resolve in my own mind, and which I think might possibly actually turn out not to be "discrepancies" at all but guide posts pointing in *another* direction, possibly eventually to be found to be the right one. *I admit that much of my unwillingness to accept vaccinotherapy at its apparent face value in blastomycosis stems from two sources:* first, the deep seated

conviction that the similar type of therapy is probably useless and possibly even slightly harmful when applied in *coccidioidomycosis;* and second, (in reality a corollary to the first,) the realization that the more complete our understanding has become, the more points have come to light in which certain of the deep fungous diseases behave similarly, among which are several instances which cause coccidioidomycosis and blastomycosis to appear to be parallel.

It will facilitate the following discussion therefore to review first briefly the situation as regards vaccinotherapy in coccidioidomycosis. Coccidioidin, no matter *how* it is prepared, is an incomplete antigen; that is it cannot produce *any* demonstrable antibodies when injected into *normal* animals or human beings which could then later react with it in a testing procedure. This is an advantage in one way, since it need not be withheld for fear of causing future reactivity which could interfere with its specific usefulness in prognosis; but on the other hand it makes it difficult to accept the possibility that it could act as a "vaccine," in which capacity it would almost certainly *have* to produce antibodies which would be effectively helpful to the patient in *resisting* the disease. Especially significant is the fact that it cannot induce the reactivity to the *skin test* which clinicians have observed to parallel closely the level of immunologic resistance. In fact the very reverse is the true situation, since coccidioidin *can* lower the degree of skin test reactivity possessed by a patient by the "desensitization" process, thereby being subject to suspicion of being able to *lower* his level of immunologic resistance. Coccidioidin is however low in nitrogen content (3%), very low in amino acid structure (0.6%) and extremely low in protein elements (0.09 mg per cc.) It has always been maintained by some workers

(Jacobson and Stewart) without confirmation by others that perhaps some methods of preparation might be discovered which could increase this protein like fraction sufficiently to enable the material to act as a vaccine.

In contrast to *Coccidioides immitis, Blastomyces dermatitidis* apparently produces a *great deal* of protein-like material, at least in its yeast-like culture phase at 37° C. Martin *does not state the percentages in the several fractions* he obtained by precipitation with ammonium sulfate but he described a "copious precipitate" produced by the addition of trichloracetic acid to the filtrate of the original material after sonic treatment of the cells. *Of* Martin's two types of blastomycin, the one he obtained *first (by washing the yeast-like cells with saline before* sonic treatment) was classed as a pure polysaccharide *giving none of the usual tests for protein. (Not, however* shown to be *nitrogen* free, nor *amino* acid free, nor electrophoretically *protein free). This* substance would seem *to be closely similar to coccidioidin and hence might be* presumed to equally lacking in antigenic ability of the type needed for it to be able to act successfully as a vaccine. His second material contained most of the protein structure, along with a considerable polysaccharide component. It cannot be denied that this *second* material might prove to be antigenic, and even able to produce specific antibodies helpful in assisting the body to resist blastomycosis. *In fact, the conjugation of polysaccharide* components with protein components as it occurs in this *naturally* obtained substance may be the "complete antigen" corresponding *to that which it was hoped could* be produced by coupling the incompletely antigenic *coccidioidin* to other substances.

However, the *vaccine* usually recommended for blastomycosis has been that obtained by simply killing the en-

tire yeast phase (grown at 37° C.) of *Blastomyces* cultures by heat. This material must contain *both* of Martin's fractions, the polysaccharide as well as the protein-polysaccharide components. The *former* might be suspected of "desensitizing" the patient to his skin test reactivity and thereby reducing his immunologic resistance, even if and while the latter substance was *stimulating* it. Furthermore, heating coccidioidin separates some of its nitrogen and thereby reduces its non-polysaccharide (protein-like) component; thus, treating blastomyces vaccine by heat may also *reduce the very component* which might be of use in stimulating the development of immunologic resistance. It is noteworthy in this regard that the advocates of vaccinotherapy recommend a "heat killed yeast phase vaccine." It would seem that Martin's second fraction (protein + polysaccharide) would be better. Of course there is always the possibility that the *cell walls* of the blastomycetes are actually the immunity producing factor, and that no *soluble* extract can duplicate the results which follow the actual presence of the infection.

Even if all of the foregoing optimistic possibilities are actually true, however, it seems likely to me that "vaccinotherapy" is destined to failure on theoretical grounds except under circumstances peculiar to one type of case. As was extensively discussed under coccidioidomycosis (see page 67), it is difficult for me to accept the belief that *small* amounts of killed organisms grown in culture can engender an effective immunologic change in an individual obviously unable to respond in a similar manner to *myriads* of *live organisms* engaged in actively producing the disease within his body It is an acceptable thesis that such material could confer upon a *normal* person an effective degree of immunity if injected *before* infection took place, but I think it highly unlikely that this mech-

anism can work *after* an extensive infection has already been established.

There is, however, the one circumstance previously mentioned which may still make success possible. If we postulate that in the chronic cutaneous form of blastomycosis (known to contain but a paucity of the organisms, and these all very well shielded from the rest of the body by an intense granulomatous and cellular infiltrate) the oragnisms and their products are not brought into sufficiently close contact with those organs or cells in the patient's body whose duty it is to produce the specific antibodies which confer immunity, then it is possible that injections of *killed* organisms where they CAN approach these organs or cells *might* function in an effective manner. It must be recalled, however, that patients possessing only this chronic cutaneous type of blastomycosis usually already possess a high degree of skin test reactivity as well, indicating according to our present view a high degree of immunologic resistance. Just what *additional* elements of resistance might be stimulated by vaccine under these circumstances must be speculative at present. It must also be wondered whether, if certain factors can succeed in isolating the causative organisms in such a manner as to prevent their stimulating the host's resistance mechanisms, these same factors might not also hinder the reverse of this process, and effectively prevent any antibodies as might be produced in response to the injection of an artificial vaccine from being brought into destructive contact with the fungi.

It would also be valuable to know whether or not the killed "yeast phase" vaccine is capable of acting as an antigen in a demonstrable way *in normal human beings;* that is, by inducing the ability of the body to react subsequently to one of the tests with blastomycin Particularly

impressive would be the ability to render normal persons reactive to the *skin* test in high degree, since this form of reactivity appears to parallel immunologic resistance. The advocates of vaccinotherapy have not made this claim nor even that such a change takes place in persons already harboring blastomycosis as the *result* of such therapy, but there is at least an implication that this is the fact in such statements as "blastomyces vaccine was administered during succeeding weeks, after which the intradermal reaction to blastomycin was positive and the complement fixation test was positive in low titer."

The reader should not infer however that this vaccine must of necessity be considered worthless if it should prove to be unable to induce in normal persons the ability to react with blastomycin It is certainly *possible* that immunity might be attained without such skin test reactivity being developed, since in the light of our present evidence it is far from certain that they are inseparable or due to the same factors completely, if indeed they are at all It is only clear that in the course of the *natural* history of the disease they *parallel* each other to a degree which is useful to the *clinician* in arriving at the *prognosis*.

Surgical Intervention

Surgical intervention by cold knife excision has been frequently followed by recurrence, probably due to the reimplantation of the organisms into the wound. Some cases of pulmonary blastomycosis have proceeded to cure after lobectomy or pneumonectomy, but it is difficult to decide what the outcome would have been without it because determination of the reactivity to the blastomycin tests was not included in the reports. In the localized, chronic cutaneous form, the disease can be markedly im-

proved and even cured by thorough *curettage* followed by *desiccation*, a procedure which is accomplished with surprising ease since all involved tissue separates without much resistance along a well defined cleavage plane, leaving an almost completely uninfected, healthy base. Subsequent use of Lugol's compound solution of iodine, diluted with an equal part of water, as a daily dressing appears to assist in preventing recurrences; and any such which develop can be easily curetted again while still small. The recent concept that this form of blastomycosis represents the end stage of a process of dissemination in which all body tissues other than the skin have long ago healed and thereafter possess an adequate degree of resistance of a type which probably persists, removes to a large extent the reluctance to attack such lesions surgically because of fear of disseminating the organisms. Also, since the source from which the skin originally acquired its infection by dissemination (usually pulmonary) has become completely cleared in many of the cases of more than a year or two in duration, it is unlikely that the skin can subsequently acquire a new infection from within after it has once completely healed

X-radiation

X-radiation has been employed in many cases of chronic cutaneous blastomycosis during past years and has frequently been followed by a dramatic degree of improvement in the lesions Seldom has *complete* cure by this means alone been claimed, however, a fact which seems to be of extreme importance in understanding its action. When one recalls that the great mass of the diseased tissue in this type of infection is composed of cells contributed by the host, principally leucocytes and hyperplastic epidermal cells, with only an extremely rare blas

tomycete, it is logical to conclude that x-radiation could hardly fail to cause considerable shrinkage by its deleterious direct action on such radiosensitive *host* cells. It appears even possible that this procedure might even be detrimental in the long run by removing some portion of the host's cellular barriers to the spread of the disease.

Derivatives of Stilbene

Stilbamidine, hydroxystilbamidine and certain other derivatives of stilbene have been extensively employed since the original observation by Elson that they could exert a specific chemotherapeutic action. A large number of similar compounds, most of which contain the double bonded carbon grouping of ethylene (syrene, nitrostyrene, cinnamic acid, etc.) are being investigated by Curtis and his co-workers, and some appear to be promising. Stilbamidine is as yet the most widely used of these drugs, and is administered in a dosage of 150 mg daily for 2 weeks, given slowly intravenously in 500 cc. of normal saline. This course is repeated after an interval of 2 weeks. Neurotoxicity has been troublesome, especially with regard to the trigeminal nerve and hepato-toxicity has been encountered in serious degrees Hydroxy-stilbamidine is reported to be less toxic. The clinical response is much better in the chronic cutaneous form than in the pulmonary or disseminated types This might indicate that the efficiency of these drugs is not due simply to antifungal action, but to some enhancement of immunologic processes, which are probably already well developed in the cutaneous type. It is also true that some of these investigations lack the control element which the persistent employment of the skin test and complement fixation reactions could furnish by determining the prognosis of each

case if such drugs were *not* used. The status of this type of therapy is in a state of flux at this writing and the most recent publications should be studied before any such treatment is begun.

Nonspecific Measures

Nonspecific measures should still be considered highly important because there are so many poorly understood facets of the pathogenesis of blastomycosis. Even though there is considerable doubt as to how *effective* they may be, they will not interfere with whatever more *"active"* procedures may be additionally selected. Thus, it is advisable to employ *all* those measures which may be calculated to enhance the *natural* resistance of the patient as well as those which are presumably able to assist in the development of *specific* immunologic resistance. Prolonged rest in bed, a high caloric and high protein diet, supplemental vitamins, especially some fractions of B and C, crude liver extract and transfusions of whole blood are all advisable. Steroid therapy is almost certainly contraindicated, because of probable interference with allergic-immunologic processes. Antibiotics should be withheld unless indicated specifically by the concomitance of susceptible bacteria in significant degree, since they may stimulate fungi, and can conceivably furnish fungal products which could lower resistance by "desensitization." For further discussion of the reasons for these "non specific" recommendations the reader should refer to the chapter on coccidioidomycosis on page 76.

Chapter 15

HISTOPLASMOSIS—HISTORY AND THE CLINICAL SYNDROMES

ONE OF THE most outstanding examples of the fact that rapid progress can still be and is in fact being made in medical knowledge is revealed by the tremendous advancement which the last 10 years has brought in our understanding of histoplasmosis. At the beginning of this period this disease was still considered to be a very rare (only 71 cases) highly fatal, disseminated, granulomatous infection, acquired in an obscure manner, now it is recognized that within certain endemic geographic areas enormous numbers of persons become infected, by the inhalation of dust containing spores of *Histoplasma capsulatum*, and that although they seldom become involved to a degree serious to life most of these individuals subsequently for the remainder of their lives bear evidence of one sort or another of having had the disease Through this concept, one of the most puzzling features of roentgenography has apparently at last been explained, namely, the high incidence of persons exhibiting shadows interpreted as indicating pulmonary calcification which could not be attributed to tuberculosis

It should be equally impressive, however, to point out that this spectacular rate of progress in histoplasmosis almost certainly could not have occurred without several clues derived from phenomena discovered previously by

students of coccidioidomycosis. To pursue this already well blazed trail was a comparatively simple procedure, which avoided the usual course to be anticipated in studying an obscure disease, which involves the elimination of many blind alleys. Those persons studying histoplasmosis have become increasingly well aware of this fact as they have progressed, for they have encountered almost nothing except evidence tending to confirm the concept that the two diseases are very similar In fact, it was Charles E. Smith (undoubtedly the greatest *single* contributor to our knowledge of coccidioidomycosis) who first suggested that *histoplasmosis* might be found to be the cause of much nontuberculous pulmonary calcification in the Middle Western United States.

By applying these concepts, histoplasmosis has been already found to resemble coccidioidomycosis in the following facets: (a) the reservoir of the fungus in nature; (b) the manner in which it is acquired by human beings; (c) the existence of the disease in two forms, one widespread and mild, the other rare and dangerous to life; (d) the usefulness of the skin test in ascertaining which individuals have or have had the disease, and (e) the usefulness of the skin test in determining the distribution of the disease over the globe and in outlining certain endemic areas. The present author believes that *further* detailed study will reveal *still more similarities*, some of which are already *suggested* by presently recognized trends, but *not* as yet *proved*, such as the value in prognosis of skin-tests and complement fixation examinations, and the nature of the primary skin complex.

Histoplasmosis was discovered in Panama in 1906 by Darling while searching for Leishman-Donovan bodies in necropsy material from a person whose lungs, spleen, liver and lymphnodes grossly revealed pseudo-tubercles and

focal necroses. He saw the tiny (3 to 5 microns) round or oval bodies surrounded by refractive capsules and realized that they were different from *Leishmania*, but nevertheless he apparently accepted their protozoan nature. He named the organism *Histoplasma capsulata*, and this designation persists today except for the correction of the species epithet to *capsulatum* for language reasons. He reported two other cases of similar nature in 1908 and 1909. No other cases were discovered until 1925, when Riehl reported one from Austria, followed the next year by cases discovered in Central America, the Philippines and the United States (Minnesota), and still later from the Dutch East Indies. All of these authors were still doubtful of the nature of the organism, although as early as 1912 da Rocha-Lima had suggested that it might be a fungus

Working independently, De Monbreun almost simultaneously with Hansman and Schenken, in 1933 succeeded in culturing the organism and establishing its identity as a fungus; they described its characteristics and added to the ease with which the diagnosis could be established Thereafter cases were discovered with increasing frequency, so that in 1945 Parsons and Zarafonetis were able to assemble 71 such instances. In all of these cases the disease was extremely severe, and the diagnosis was made before the death of the patient in only a very few instances.

Up to this point the history of histoplamsosis closely parallels that of coccidioidomycosis before Gifford and Dickson's discovery that the latter disease exists in a widespread benign form The impetus to investigate the possibility of a similar phenomena operative in *histoplasmosis* arose from the desire to explain the presence of pulmonary calcification in large numbers of persons who could not be shown to have had tuberculosis. Nontu-

berculous pulmonary calcifications in California had been successfully attributed to coccidioidomycosis in some persons in spite of the fact that the disease had been otherwise inapparent to them throughout its course. Accordingly a number of persons who had resided in the middle western portion of the United States and who revealed pulmonary calcifications were tested with coccidioidin; many were found to react positively, but only if *strong* concentrations of the antigen were employed. *Still*, the percentage which *did* so react was found by Nelson and Furcolow to be significantly higher among those persons who *revealed* pulmonary calcifications than among those *who did not, yet none of these could be shown to have had coccidioidomycosis.* Charles E. Smith then suggested that this might be best explained on the basis of cross reactivity because of a common antigen of lessened potentiality possessed by TWO DIFFERENT FUNGI, the *second* of which was actually the cause of the disease in question. On the basis of a significant degree of localization of cases of fatal *histoplasmosis* into certain geographic areas in which the percentage of pulmonary calcification was also high, Smith suggested that in this instance the pathogenic organism might be *Histoplasma capsulatum.*

Extensive studies then began to be carried out in large segments of the population using an extract of cultures of *H. capsulatum* as a skin testing material, and the true status of this disease began to be appreciated. After *a great deal of study by* Christie and Peterson, Palmer, Prior and Allen, and others, the evidence indicated that not only was it quite *plausible* to attribute the pulmonary calcification phenomenon which was then under investigation *to histoplasmosis, but in addition it was revealed* that within certain geographic areas a very large percentage of individuals who had no such *lesions*, and in

fact gave no history even remotely suggestive of histoplasmosis, nevertheless *reacted strongly* to this test. It then began to be intensively suspected that histoplasmosis exists in a benign form which is acquired by huge numbers of persons without their ever exhibiting any symptoms or signs. This is, of course, the exact counterpart of coccidioidomycosis as we now understand it. It is probably still too early to state that this concept has been *conclusively* proved in histoplasmosis but each recent year has brought additional confirmatory evidence, until at present eventual proof seems assured. Those students who have been previously conditioned by experience with coccidioidomycosis are usually more easily convinced of the truth of the thesis The difficulty of course lies in establishing *conclusively* that the disease is actually *histoplasmosis* in patients who remain entirely asymptomatic or are never more than sightly ill. In mild cases such as these, cultural methods are difficult because organisms are not present in large numbers, and pulmonary tissues seldom become available for biopsy or autopsy study.

There is, however considerable evidence to support this view It will be recalled that it has become established that a *conversion* of the skin test response to coccidioidin from negative to positive during the early stages of a respiratory infection can be accepted as indicating that the disease is coccidioidomycosis. In the same manner the histoplasmin test has frequently been observed to convert; for example seventeen such conversions were noted by Furcolow, Guntheroth and Willis, only seven of which patients exhibited any symptoms or roentegenographic signs Similarly Nilzen and Paldrok found that seven persons who became ill after handling cultures of *H capsulatum* developed positive skin test reactivity to histoplasmin Christie has demonstrated this fungus in

berculous pulmonary calcifications in California had been successfully attributed to coccidioidomycosis in some persons in spite of the fact that the disease had been otherwise inapparent to them throughout its course. Accordingly a number of persons who had resided in the middle western portion of the United States and who revealed pulmonary calcifications were tested with coccidioidin; many were found to react positively, but only if *strong* concentrations of the antigen were employed. Still, the percentage which *did* so react was found by Nelson and Furcolow to be significantly higher among those persons who *revealed* pulmonary calcifications than among those who *did not*, yet none of these could be shown to have had *coccidioidomycosis*. Charles E. Smith then suggested that this might be best explained on the basis of cross reactivity because of a common antigen of lessened potentiality possessed by TWO DIFFERENT FUNGI, the *second* of which was actually the cause of the disease in question. On the basis of a significant degree of localization of cases of fatal *histoplasmosis* into certain geographic areas in which the percentage of pulmonary calcification was also high, Smith suggested that in this instance the pathogenic organism might be *Histoplasma capsulatum*.

Extensive studies then began to be carried out in large segments of the population using an extract of cultures of *H. capsulatum* as a skin testing material, and the true status of this disease began to be appreciated. After a great deal of study by Christie and Peterson, Palmer, Prior and Allen, and others, the evidence indicated that not only was it quite *plausible* to attribute the pulmonary calcification phenomenon which was then under investigation to histoplasmosis, but in addition it was revealed that within certain geographic areas a very large percentage of individuals who had no such *lesions*, and in

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cervical lymph glands from children who subsequently recovered, and in the lungs of children dying from other causes, in whom the histoplasmosis itself had been inapparent. Straub and Schwarz found evidence of past histoplasmosis in the lungs of 67% of persons dying from other diseases within an "endemic" area.

Furcolow and Grayston have studied a number of instances in which groups of persons have been exposed to dust under certain similar circumstances. A high percentage of these persons have become infected with what is almost certainly histoplasmosis. These "epidemics" have been very instructive, since the fungus itself has frequently been recovered in artificial culture from the source of the dust, often apparently significantly associated with the presence of chicken or pigeon manure.

There is also much confirmatory evidence derived from the study of proved histoplasmosis naturally occurring in animals, the incidence of which follows the same general geographic distribution as that of cutaneous reactivity to histoplasmin in human beings. Involvement has been reported in dogs, cats, rats, mice, cattle, ferrets, horses, bears, raccoons, opossums, foxes and skunks; and many of these reports record animals which revealed significant cutaneous reactivity to histoplasmin.

This entire historical sequence has been recently most admirably presented in concise form and with exceptional conservatism by Silverman, Schwarz, Lahey and Carson.

Clinical Syndromes

Although the existence of the following syndromes as separate clinical entities cannot by any means as yet be regarded as established, there is enough indication from the recent trend to warrant discussing histoplasmosis un-

der the same classification headings as those used here for coccidioidomycosis, sporotrichosis and blastomycosis, if for no other reason than to bring all of these diseases into direct comparison.

Primary Cutaneous Histoplasmosis

In the light of the recent reports indicating the likelihood that when coccidioidomycosis and blastomycosis are acquired by a primary inoculation into the skin the resulting syndrome consists of a chancriform primary lesions accompanied with regional lymphantitis and lymphadenopathy closely resembling the ordinary type of sporotrichosis, Curtis and Harrell believe they have observed the analagous picture in histoplasmosis. Their patient exhibited an ulcerative lesion on the penis, accompanied by regional lymphadenopathy; the disease was conclusively proved to be histoplasmosis. He became well spontaneously, and subsequently sustained no recurrence. These authors are convinced that in this case the infection was acquired by primary cutaneous inoculation. There have been no other similar cases reported, but this need not be an argument against acceptance of this concept, since such an event may be expected to occur but rarely, because *Histoplasma capsulatum* in its cultural phase in nature resembles *Coccidioides immitis* more closely than it does *Sporotrichum schenckii*, and hence probably will not frequently become inoculated intracutaneously (see also the discussion on page 91).

There are, of course, numerous instances in which the *first* evidence of the existence of histoplasmosis has been a lesion or lesions in the mucosa or the skin, but there is no conclusive proof that these sites were actually the portals of entry; frequently the lesions have been

identical in their morphology and subsequent course with those observed in other individuals in whom they were *known* to have occurred by hematogenous dissemination of the organisms (see also under coccidioidomycosis, page 15, and blastomycosis, page 109). Histoplasmosis has, however, been produced by direct inoculation of the organisms into the mucous membranes or the skin of animals, and a similar route of infection in human beings must be considered possible. If and when the question of intracutaneous inoculation arises in the future, students of histoplasmosis would do well to attempt to apply the criteria outlined for primary cutaneous coccidioidomycosis outlined on page 13. There is insufficient evidence at this time to allow this phase to be discussed further here.

Primary Pulmonary Histoplasmosis

As has been said, there is as yet no incontrovertible evidence which will allow us to separate a group of cases of histoplasmosis observed to be limited to the lungs and establish them as a clinical entity separate from other manifestations of the disease, but it seems virtually certain that such a division will be found to be valid. This group would include all those cases which remain entirely subclinical and asymptomatic throughout the entire course of the disease and clear quickly, leaving as sequelae only the ability to react positively to the skin test and in a percentage of instances to exhibit pulmonary or splenic calcification shadows by x-ray examination Also it should probably include the early phase of most of those cases of histoplasmosis in which the disease was shown to be present first in the lungs, even though it later spread by hematogenous dissemination to involve other organs Bunnell and Furcolow observed a proved case in which the

disease remained limited to the lungs and was followed by recovery. Other cases apparently limited to the respiratory system have been repotred by Christie. Schulz found the lungs to be the most frequently involved organ in persons above the age of 5 years, below this the disease favored the liver or lymph nodes. Straub and Schwarz found evidence of past or present histoplasmosis in the lungs of 67% of 105 persons dying of other causes within an endemic geographic area.

Hodgson, Weed and Clagett have described the symptomatology of pulmonary histoplasmosis, although they did not limit their description to the *primary* pulmonary type of involvement. Cough with or without sputum is common, chest pain is frequent, fever is the rule, hemoptysis is rare. Dyspnea and noctidrosis occur. Ulcerated lesions of the larynx have been described, with hoarseness as the presenting symptom. Atelectasis, emphysema, cavitation or pleurisy with effusion can occur and produce their typical symptoms.

Schulz has described pathologically the primary pulmonary complex of histoplasmosis as being similar in many respects to that of tuberculosis in size, shape, and location. Usually it is a small, circumscribed subpleural lesion, generally caseated, more frequently in the lower two-thirds of the lobes. The regional lymph nodes are hyperplastic, but less likely to be caseous than in tuberculosis.

There are no diagnostic radiographic features by which histoplasmosis may be differentiated from other pulmonary inflammatory disease, and the picture varies extensively. Primary complexes have been shown at autopsy which were not revealed by chest films taken only the day before death (Silverman, Schwarz, Lahey and Carson).

The incubation period as established by study of the epidemics is said to be from 5 to 15 days.

Disseminated Histoplasmosis

The utilization here of the term "disseminated" for the type of histoplasmosis known for so many years in which the disease involves many parts of the body, is subject to some criticism, since it implies that there is conclusive proof of the existence of a preceding "primary" form of the infection. Since the trend of our advancing knowledge is ever more strongly in this direction, however, it seems virtually certain that this concept will soon become firmly established as fact; it is therefore considered *appropriate to place histoplasmosis in apposition to those* deep mycoses which have been previously discussed in this volume, by naming and discussing the clinical syndromes in a similar manner.

In conformity with this thesis, it is postulated that a *certain very small percentage of the vast number of persons* who acquire the primary pulmonary form of histoplasmosis fail to resist it in the usual efficient manner, sustain more extensive involvement of the lungs and finally the fungous cells are permitted to enter the blood stream and be carried throughout the body to lodge in the capillary beds Lesions which have proved to be histoplasmosis have been reported in all organs of the body except the cortex of bone and cartilage, but not all tissues are equally susceptible, and there can be considerable variation in the resulting disease picture.

There is reason to believe that even following dissemination some individuals can still resist the disease sufficiently to keep it entirely asymptomatic while they achieve a natural cure, since areas of calcification simi-

lar to those so well known in the lungs, are sometimes found by roentgenography in other organs also, especially the spleen, in otherwise "normal" individuals residing in so-called "endemic areas" (Straub and Schwarz). Also, as has already been mentioned, a high percentage of persons dying from entirely unrelated causes and without any symptoms suggesting histoplasmosis, have nevertheless been shown at autopsy to have harbored the infection (Christie and Straub and Schwarz (1950). However, the majority of patients with *proved* disseminated histoplasmosis have become seriously ill, and a fatal termination has usually ensued, although sometimes only after several years.

H. capsulatum shows a pronounced predilection for invasion of all types of cells belonging to or derived from the reticuloendothelial system; sometimes this appears to be due to a preceding disease of this system, at least it has been established that histoplasmosis and such reticuloendothelioses as leukemia, Hodgkin's disease, lymphosarcoma and sarcoidosis are found to be coexistent much more frequently than is statistically justifiable on the basis of coincidence.

Radaelli and Ciferri have shown that elements of the fungus which are introduced into animals are phagocytized at once by monocytes and macrophages, in which they do not die but multiply and are subsequently borne hematogenously or by way of the lymphatics to organs rich in reticuloendothelial elements such as the lymph nodes, spleen, liver and bone marrow This pronounced predilection for a certain type of cell largely determines the clinical picture which follows in the typical case, although it often spreads beyond any such confines, and can result in a wide variety of signs and symptoms.

The disease progresses at variable rates. The usual syndrome is that of a subacute to chronic wasting, irregularly febrile disease, accompanied usually by anemia, leucopenia, generalized lymphadenopathy and enlargement of the spleen and liver. The symptoms commonly encountered are nausea, vomiting, anorexia, diarrhea and gastrointestinal pain. Pleurisy, pulmonary infiltration and tracheobronchitis occur frequently; and endocarditis, involvement of the adrenals, or central nervous system are reported occasionally. Lesions of the mucous membranes are common, especially of the mouth, tongue and genitalia, usually in the form of torpid ulcers or granulomatous masses accompanied by induration. Similar lesions may occur in the skin, where purpuric or even bullous eruptions are occasionally encountered.

Although disseminated histoplasmosis occurs at all ages, there is a pronounced increase of incidence in the very young and very old. In infancy and childhood it is insidious in its onset, and seldom furnishes any mucous membrane or cutaneous lesions as clues to the granulomatous nature of the process and as sources of biopsy material. Histoplasmosis lacks the preponderant association with male manual laborers to the extent evidenced in some of the other deep mycoses, but those persons whose work exposes them to soil or avian excreta appear more likely to become infected. In childhood both sexes are about equally affected.

Pathology

Primary pulmonary histoplasmosis has not as yet been adequately studied from the pathological viewpoint due to the mild nature of the disorder and the consequent rarity of post-mortem material. This phase of the subject can-

not be discussed further than has already been done under the heading of clinical characteristics.

Disseminated histoplasmosis has been very well delineated, however. The essential lesion is a granulomatous nodule which tends to ulcerate. The lungs are almost always involved, revealing miliary tubercles, consolidations resembling tuberculosis and hilar lymphadenopathy. The bone marrow is almost always involved. The spleen and liver are enlarged, and hyperemic, and show tubercles and necrotic areas The adrenals may be involved, and less frequently almost any other tissue of the body except cortical bone and cartilage.

Histopathologically it is strikingly evident that the fungus is seldom seen extracellularly, it is an "endoparasite" of cells, preferring usually those of reticuloendothelial origin It appears as a round or slightly oval body, from one to three microns in diameter which appears to be surrounded by a "capsule" (Pillsbury and Kligman have recently demonstrated a cell wall peripheral to what has been called the "capsule") Occasional cells are seen in the process of producing a single bud.

Granulomas of the pseudo-tuberculoid type develop around masses of parasitized cells, consisting of caseous material surrounded by a zone of granulation tissue composed of lymphocytes, plasma cells, fibroblasts, macrophages, epithelioid cells and giant cells Coalescence of such foci can produce large necrotic areas Sometimes evidence of healing is seen in the fibrosis This lends weight to the thesis that some mild cases may recover without being recognized.

In addition to the evidence for the existence of histoplasmosis which may be obtained from the clinical picture and the skin test and complement fixation titer the diagnosis can be confirmed occasionally by histopathologic

preparation of biopsy material, by directly examining *microscopically smears of circulating blood (especially the* buffy coat), or bone marrow, by culturing the organisms from any of these sources as well as from sputum, and by intraperitoneal inoculation into mice.

Chapter 16

HISTOPLASMOSIS—IMMUNOLOGIC AND THERAPEUTIC CONSIDERATIONS

ACCEPTING, for the moment at least, the thesis that a large percentage of persons residing within certain geographic areas become infected with histoplasmosis by inhalation of the spores of the fungus and that they thereafter sustain a pulmonary disease which is almost always mild and frequently unnoticed in its usual course toward complete recovery, it is of the greatest importance to consider the reasons why a certain very small number of such individuals do NOT so successfully fight the disease but instead become heavily and extensively involved with a form of the infection highly dangerous to life. It will be recalled that this question arose similarly in the preceding chapters in connection with coccidioidomycosis and to a lesser degree with sporotrichosis and blastomycosis, and that only two theories were advanced as possible explanations, that the answer might lie in the massiveness of the original inoculation of fungous elements, or that these certain individuals possess at the time of, or before the infection, or develop after it an important defect in the mechanisms by which the vast majority of their fellow sufferers so easily acquire specific immunologic resistance Our present knowledge of these three diseases does not permit us to do more than vaguely state such

suspicions since there is absolutely no evidence to confirm or deny them.

It has been frequently pointed out that histoplasmosis is associated with other granulomatous disorders, especially tuberculosis, more often than coincidence would indicate, as is also the case with other deep mycoses. This observation is subject to several possible interpretations, among which our present knowledge does not permit us to discriminate; the presence of tuberculosis may cause a superimposed histoplasmosis infection to be resisted poorly and thus disseminate; or the two diseases may be reversed in sequence; or the two diseases may simply act synergistically; or the same inherent defect in the particular patient's immunity mechanisms may similarly and simultaneously allow *both* diseases to develop into serious forms. These same theories are, of course, equally applicable to all other instances in which two disorders are found in conjunction.

However, *histoplamsosis* furnishes some additional valuable clues to the solution of this problem. First, *there have been several "epidemics" of histoplasmosis* caused by the inhalation of large quantities of dust heavily contaminated with *H. capsulatum*, so that the resulting inoculation must have been tremendously more massive than that inhaled by the average individual who acquires the infection; yet the percentage of cases in which *dissemination* occurred seems not to be significantly higher in this group This appears to negate the importance of the massiveness of the original inoculum. Second, there have been altogether too many instances in which disseminated histoplasmosis has been found in association with some form of disease of the reticuloendothelial and hemopoietic systems to be accepted as coincidental. *It is also probably significant that there has been no strict*

uniformity in the *type* of disease found thus associated, various forms of leukemia, lymphosarcoma or Hodgkin's disease having been implicated.* A logical explanation is that the lymphoblastomatous *disease* process causes the reticuloendothelial system to fail in some manner in the performance of what is considered by many students as one of its most important *duties*, that of the production of some specific antibody or antibodies necessary for the development of *immunity*. The reverse of this thesis is also *possible*, although it seems more remotely so, that histoplasmosis may induce changes in the reticuloendothelial system which *result* in a syndrome indistinguishable from one of these lymphoblastomata.

There are some enticing fields for study here For example, could the cells of tissue cultures of reticuloendothelial elements derived from individuals fatally involved with histoplasmosis be shown to be more susceptible to intracellular invasion by the fungus than those from normal persons? From persons known to have recovered? From persons recalling no illness of the histoplasmosis type but nevertheless able to react to intracutaneous histoplasmin? Could serum or plasma from patients who have recovered from histoplasmosis inhibit this susceptibility? If so, then could similar material taken from persons who recall no significant disease but react positively to histoplasmin accomplish the same purpose? Some clues to the nature of immunity might be discovered by such investigations, particularly whether susceptibility and immunity are functions of *serum*, or of any of the various types of *blood cells* or *fixed tissue cells* Work of this sort would of course be extremely difficult, but should be equally

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*Wilkerson saw statistical correlation of disseminated histoplasmosis even with *carcinoma*, both in man and in cattle.

clearly decisive and rewarding. The implications of its value toward the development of our understanding of the immunology of infectious diseases in general are tremendous.

Of all infectious diseases, histoplasmosis is at present the most enticing for study, possessing as it does this phenomenon of statistical relationship with diseases of the reticuloendothelial system, so fortuitously combined with epidemiologic, clinical and immunologic manifestations so closely similar to those of coccidioidomycosis from which we have already derived so many valuable clues. No other disease is so well endowed with such a complete constellation of such potentially important and clearly visible phenomena.

It is very important to determine accurately wherein histoplasmosis duplicates the activities of coccidioidomycosis, and wherein it differs, thus potentiating or diminishing the degree of faith which we can accord to the presently held theories. I believe it is fair at the outset to state that there is as yet no *irrefutable* evidence at any point that there are differences of major importance. At the same time, it must be admitted that there are many discrepancies which must be explained, as well as gaps in our knowledge which will have to be filled before it can be accepted *conclusively* that the trends are identical. In the preceding chapter reference has been made to similarities in regard to the reservoir of these two fungi in nature, the manner in which the two diseases are acquired by man, the existence of each disease in two forms, one widespread and mild followed by recovery and subsequent immunity, the other rare and dangerous to life, the usefulness of a skin test in ascertaining which individuals have or have had the disease, and in determining the distribution of the disease over the globe and in outlining

certain areas as endemic. Most of these facets seem destined to become firmly established as factual, although additional confirmatory evidence is desirable.

The Histoplasmin Intracutaneous Test

However, these observations do not exhaust the possibilities of similarities in the two diseases. The intracutaneous test utilizing histoplasmin seems particularly promising in this regard, at least to those who accept the thesis that a positive reaction of the *delayed tuberculin type* to the intracutaneous coccidioidin test indicates that that individual possesses immunologic resistance against coccidioidomycosis.* The most impressive bit of evidence supporting this contention is, of course, the fact that such tremendous numbers of persons exist who show such reactivity to intracutaneous histoplasmin, but who are entirely well, and indeed have remained unaware of the infection throughout its course and subsequently, even though many of these persons reveal x-ray evidence that it was present in a fairly *extensive and severe form*. Of course, we have no way of knowing whether or not some persons who do *not* react positively to the skin test may not *also* have had the disease and recovered and may *also* be subsequently immune, except for the fact that in such individuals evidence of pulmonary calcifications such as are presently attributed to old histoplasmosis do not occur with statistical significance

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*As early as 1941, Van Pernis, Benson and Holinger had noted an "immediate flare" reaction when histoplasmin was intracutaneously injected They attached no clinical significance to this observation There has been little comment on this phase of reactivity in subsequent literature, and its status at this time is the same as in the case of coccidioidomycosis, i e , there is no clinical significance which can be as yet attached to this type of response (see also under coccidioidomycosis, page 58)

How sure can we be that the ability to react to the histoplasmin skin test is acquired only through a specific process in response to an infection with *H. capsulatum*? There are studies (notably that of Furcolow, Willis, Wood and Mantz) which indicate that for each geographic area there is a consistent rate of "conversion" of the ability to react to the test from negative to positive for each year of residence in the area, averaging about 3% annually for the first five years of life, and about 8% annually *thereafter totalling some 70% at about age 20.* The slower rate in infancy is probably correlatable with the higher incidence of *fatal* histoplasmosis in this age group, and supports the view that a positive reaction is associated with immunity. That this "conversion" is a specific process and almost certainly due to histoplasmosis can be inferred from the fact that 59% of a group of such persons also showed positive serologic reactions to histoplasmin, and that 19% revealed typical active foci and 13% healing foci by roentgenographic pulmonary studies. However doubtful the above conclusions are at present, we *are* privileged to say that a positive skin reactivity *is* commonly found in many persons who are entirely well even though probably *previously infected* and to imply that there *may* be a cause and effect relationship.

On the other hand, we know that many individuals who have *serious disseminated histoplasmosis* do *not* react to the histoplasmin skin test. Whether they could have done so at some earlier stage of the disease only to lose this ability later is presently not clear. Thus, we have two ends of a potential "measuring stick" which resembles the one proposed for the clinician's use in coccidiodioidomycosis; the intermediate markings are not as yet well established, but must wait additional experience. There are many indications however that the titer declines as re-

covery takes place. This needed evidence can most easily and rapidly be accumulated if histoplasmin testing is employed *at frequent intervals* during the course of a number of cases of histoplasmosis instead of only once as is presently the custom, and also if various dilutions are simultaneously used in an effort to determine whether this reactivity is subject to quantitation by a sort of "titration," as has been done with coccidioidomycosis.

It is not well established how long the ability to react to the histoplasmin skin test is retained, once it has been acquired. In studying one of the mass infections (called "epidemics") known to have occurred in 1941, Salvin, Furcolow and Nishio found that all 22 of the patients who were tested reacted positively 8 years later. It seems certain that the high *percentage* of positive reactors actually found to exist in certain geographic areas could *never* have been attained if the reactivity did not persist at least for many years However, several investigators have commented upon the fact that in the higher age brackets the percentage of positive reactors declines, indicating the probability that the reactivity does become gradually lost in old age or after a great many years.* All of these facts are consistent with the currently held views with regard to coccidioidin skin testing

The histoplasmin skin test has been studied extensively by several groups of workers, Van Pernis, Benson and Holinger (1941), Zarafonetis and Lindberg, (1941) Christie and Peterson (1945,) and Palmer (1945) were among the earliest Standardization of histoplasmin by Shaw, Howell and Weiss in 1950 was a valuable effort,

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*Could the 'loss of reactivity' thus postulated cause such persons to again become susceptible after long years of immunity? This *might* be the reason for the increase in incidence which has been observed in the "very old"

this material is usually called "Howell's H 15." The material used has usually been obtained in a manner closely duplicating that for coccidioidin, that is, by growing *H. capsulatum* at room temperature for several months in the same synthetic medium as that used for *Coccidioides* (see page 43). Various workers have used dilutions of either 1-1000 or 1-100.

The specificity of the histoplasmin skin test as used thus far is reputed to be not as high as that of coccidioidin, there being evidence of cross reactivity with patients possessing other disorders, notably blastomycosis and coccidioidomycosis; but most observers have concluded that *the reaction will take place with a significantly higher* dilution of the truly *specific* material, so that these false reactions can usually be easily ruled out of consideration. Some of these nonspecific or false positive reactions might also be eliminated by further purification of histoplasmin, as has been suggested for the skin testing antigens for the other mycoses heretofore discussed. Heating does not injure the *skin* testing efficiency of coccidioidin while it *eliminates some proteinlike constituents which might be* somewhat responsible for lowering the specificity by causing non specific protein reactions. Cross and Howell have isolated an immunologically active polysaccharide from histoplasmin which was found to be "protein free," although *still containing* 0.46 mg. of nitrogen per ml. (see also page 43 for comparison with coccidioidin). This substance might well afford a higher degree of specificity.

It is thus evident that there is still no unanimity of thought as to histoplasmin testing, either with regard to the ideal type of testing material, its appropriate dilutions, *or the interpretation of the results* In the opinion of this author, however, there is still sufficient indication to warrant the expectation that when these differences are

all resolved there will be found to be a *statistical relationship* between the ability to react positively to the test and the possession of effectual degrees of *immunologic resistance* as seems so well established in coccidioidomycosis. At least, it can do no harm to use this postulate as one of our prognostic tools, as has become customary with coccidioidomycosis, and future developments will test its veracity.

The Complement Fixation Test in Histoplasmosis

Although there has been a great deal of effort expended in the development of serological testing procedures in histoplasmosis, our knowledge is still not sufficient to enable us to obtain constant and unequivocal results subject to valuable interpretation and significant usefulness. There have been all too few cases of the extensive disseminated form of histoplasmosis which have been adequately studied at sufficiently close intervals to permit definite conclusions There is also still a great deal of conflict in opinions as to the proper antigenic material, and the technique of the test itself. As to the antigen, recommendations have included heat killed yeast-phase cells, formalin killed yeast-phase cells (Salvin), an extract from the broth filtrate of mycelial phase cultures (Salvin; Furcolow, Bunnell and Tennenberg; Schubert, Ajello, Stanford and Grant; and Tennenberg and Howell), and an extract from ground yeast cells (Campbell, Hodges and Hill).*

*Schubert, Ajello, Stanford and Grant have shown that the yield of complement fixing antigen and its concentration is highly variable according to which of several "strains" of the fungus is used It should be recalled that similarly considerable variation in yield was encountered by Smith in the preparation of complement fixing antigens from *Coc-*

(*Continued at bottom of next page*)

However, in spite of such confusion, most observers believe that a positive complement fixation reaction in *high titer* is associated with *serious, disseminated histoplasmosis,* and is *absent* or in *low titer* only in the *mild* form of the disease, in exactly the same fashion as in *coccidioidomycosis* There are studies such as that of Salvin, Furcolow and Nishio (1954) which indicate that complement fixing reactivity can persist for several years after apparent recovery but always in a very low titer if the patient is clinically apparently entirely well.

Experience in the coccidioidin complement fixation reaction indicates that the complement fixing ability of any antigen probably resides in a nitrogenous portion of the molecule, probably protein-like in nature, and that the best antigen should therefore be one in which every effort has been made to *avoid injuring* such delicate *protein-like components* in the process of preparation, such as by heating, drying, and chemical killing of the organisms, *as so often utilized by most present methods.* The yeast-like phase of the fungus in culture would seem to offer the closer approximation of its parasitic activities, and probably a *greater degree of specificity* (Pates, 1948, and others) Preliminary studies should be made with suspensions of *unkilled cells uninjured* in *any manner,* and then with *similar material disrupted only by sonic* vibrations (see Martin's work with blastomycin on page 129). Later, after such "standardization," comparisons can be made *with preserved antigens,* perhaps utilizing quick freezing and lyophilization techniques.

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cidioides even when the *same* strain was used, implanted at the same time, on the same medium and cultured and extracted in exactly the same manner These peculiar observations offer challenging opportunities for further investigations

It is again necessary to point out that discrepancies in the titers of *all* of these tests should not at once be assumed to indicate their *unreliability*, but rather that the reasons should be sought in subtle variations in the *clinical* status of the particular patients, hoping to discover what the test is *trying to tell us*. The *worst* example is for an observer to assume that the skin test and complement fixation reaction mean the same thing, and that when they do not react in parallel fashion one of the tests is "wrong." *There is no parallel* between these two tests in *any* deep mycosis, they reveal the presence of *entirely different antibodies* either of which can be present in *any* quantity without regard to the *other* (see discussion on coccidioidomycosis on page 58).

I believe it is likely that clarification of these features by further experience will furnish us with a complement fixation test in histoplasmosis whose titer can be used by the clinician as a measure of the severity of the disease at the time the test is performed (More probably, this will measure the quantity of *H capsulatum* organisms *actively engaged in producing the disease* at that time.) Correlation of this titer with the patient's ability to react to the skin test, indicating perhaps his level of immunologic resistance (?) should furnish us with valuable prognostic tools, even as is apparently the case in coccidioidomycosis and probably also in other deep mycoses

The Precipitin Reaction in Histoplasmosis

Van Pernis, Benson and Holinger in 1941 tried unsuccessfully to demonstrate precipitins in the serum from their patient who died of histoplasmosis, in the light of subsequent events it was probably too late in the course of the disease for them to be present Scheff in 1946 inves-

tigated the precipitin phenomenon with inconstant results.

Pates in 1948 separated from histoplasmin four fractions and found that one exhibited a higher degree of precipitation specificity than the others when tested with serum from infected rabbits; it was said to be polysaccharide in nature. Her other fractions cross-reacted also with blastomycosis rabbit serum in an undesirable manner and extent. Salvin and Hottle (1948) showed in experimentally infected rabbits that precipitins appeared in the sera during the second week of the infection and disappeared at about the tenth week. The polysaccharide-like "histoplasmin" was used as the antigen.

Because of Smith's success in demonstrating the value of the precipitin test in human coccidioidomycosis, Salvin and Furcolow (1954) studied this type of reaction in histoplasmosis. They succeeded in demonstrating precipitins in human infections as early as a week or two after the onset of clinical symptoms, and found that they persisted for periods of time varying from 3 weeks to as much as 10 months. There was no correlation between this reactivity and that of the complement fixation test, indicating that the antibodies in the sera which are concerned differ from each other. Patients who were too mildly ill to reveal a reaction to the complement fixation test nevertheless revealed precipitins in their sera. It was concluded that the precipitin test to histoplasmin is a useful tool in the diagnosis of *acute* cases of histoplasmosis, a positive reaction indicating the presence of *active disease at that moment* and probably in an *early acute phase*. In some cases the precipitins remained at their height for *only a short time* indicating that frequent repetition of the test is necessary to avoid missing the *height* of the reaction. Cross reactions with other deep mycoses

were encountered, but the response was found to be significantly greater to the homologous reaction. Thus, Salvin and Furcolow concluded that it is likely that *histoplasmosis* closely mimics *coccidioidomycosis* in yet another of the important manifestations.

Other Serologic Reactions in Histoplasmosis

Saslaw and Campbell, (1948 and 1949) have investigated a method of demonstrating specific antibodies against *Histoplasma capsulatum* in rabbit sera by using a collodion sensitization technique. Norden in 1949 used sheep erythrocytes sensitized with histoplasmin in his researches, and was able to demonstrate an apparently specific reaction. The interpretation of these phenomena and their possible correlation with the clinical course of histoplasmosis in human infection is at present not clear.

Therapy

The acute primary pulmonary form of histoplasmosis requires no therapy when subclinical or mild, and nonspecific supportive measures selected on clinical grounds suffice for the remainder. Bedrest, adequate nutrition, supplemental vitamins may enhance resistance and developing immunity. Antibiotics should be withheld unless specifically indicated for bacterial infection. Steroids almost certainly interfere with immune mechanisms.

In disseminated histoplasmosis ethyl vanillate has been reported as beneficial. Favorable results have been attributed to sulfonamides. Rapid advancement may occur soon, indicating a review of the most recent literature before therapy is begun.

Chapter 17

CRYPTOCOCCOSIS

IN THE DEEP mycotic diseases heretofore discussed it has been amply evident that the human body is able to offer considerable resistance to the invasion of the causative organisms. It is necessary only to recall that of the total number of persons who acquire coccidioidomycosis, sporotrichosis or histoplasmosis, the death rate is probably less than one per thousand In blastomycosis the statistics are less impressive at this time (but may be improved by the future discovery of a benign form in significant numbers of cases), nevertheless in the majority of instances resistance is present in a degree sufficient to preclude death from the infection.

In sharp contrast, conclusive proof of the presence of cryptococcosis in a human being indicates almost certainly that death will result from this cause. As a general rule, once the body has allowed *Cryptococcus neoformans* to produce disease, it fails completely thereafter to mobilize any resisting cellular reaction or to fight the infection by any observable immunologic mechanism.

Yet cryptococcosis is an extremely rare disease, a fact suggesting at first glance that the causative fungus must be only occasionally encountered. However, there is ample evidence that this is not true, in fact, *Cryptococcus neoformans* is probably present in or on the bodies of most persons at some time or times during their lives. It has

been cultured from normal skin and mucous membrane (Benham); it has been recovered from fermenting fruit juices (San Felice), from soil (Emmons), and from milk (Klein, and Carter and Young), and many of the strains so obtained exhibited a degree of virulence for laboratory animals equal to those from fatal human cases. Its distribution is apparently world-wide, since the disease is encountered without geographical limitations. Cryptococcosis also occurs in animals by natural means, but there has been no instance reported of a human infection derived from them, nor of transmission from man to man.

Thus there is no lack of opportunity to *contact* the causative organism, and the rarity of the disease can only be explained on the basis of the ability of the animal body to resist its *initial invasion* completely in some manner, except under unusual circumstances This is the same concept long so firmly established, for example, with regard to staphylococci and streptococci, in which it is accepted as fact that something must happen to lower the natural barriers of resistance, either locally or systemically, before an infection can occur. However, before attempting to analyze these phenomena with regard to cryptococcosis, a brief description of the disease itself is necessary

In 1894 Busse reported an unusual case of a woman infected with a yeast-like fungus, and Buschke added further details in 1895 After a few sporadic cases had been recorded, Verse in 1914 presented a case accompanied by conclusive proof In 1916 Stoddard and Cutler clearly delineated the pathology and the clinical picture and differentiated it from the other deep mycoses Freeman contributed a valuable monograph in 1931 as did Cox and Tolhurst in 1946, and Littman and Zimmerman in 1956.

The route by which the fungus enters the body is obscure, although Freeman, after reviewing the literature in 1931, believed it most likely that the portal of entry was usually the lungs. Cox and Tolhurst in 1946 supported this view, by demonstrating that the fungus could remain viable during at least 10 months of drying, and could hence be easily inhaled with dust. A history of a preceding pulmonary infection can be obtained with statistically significant frequency from patients suffering from cryptococcosis.

Direct inoculation through the skin has been accepted as the portal by many authors when reporting cases, but there have usually been features cited which make this conclusion doubtful. Sometimes the portal has apparently been the oropharynx or the gastrointestinal tract. *Cryptococcus neoformans* has been cultured from the blood of infected persons, indicating the possibility of hematogenous dissemination. In laboratory animals lymphatic spread has been observed, but not as yet in human disease. Semerak suggested that meningitis could occur by direct spread of the fungus from the nasopharynx.

Clinical Characteristics

It is not possible to divide cryptococcosis into the clinical syndromes in the manner which has been attempted for the four previously discussed deep mycoses. Although it was implied in the preceding paragraph that a primary cutaneous form could occur, proof is lacking. Certainly the "chancriform syndrome" so spectacular in the other diseases has never been described. However, it will be recalled that this picture occurs because the patient is *actively* resisting the infection by *cellular* and *immunologic* means, which usually is lacking in cryptococcosis Thus, even if direct inoculation of the skin *were*

to take place, a chanciform syndrome would probably not follow.

Although cryptococcosis is best known because of its predilection for invading the central nervous system, such involvement is often absent. The lungs are at least as frequently observed to be infected, and since they are thought to be the usual portal of entry the pulmonary form of the disease will be discussed first.

Pulmonary Cryptococcosis

There have as yet been no studies which suggest that a "primary" pulmonary form of cryptococcosis can be differentiated from a "disseminated" type in which extra pulmonary lesions have not as yet developed. The clinical picture of pulmonary cryptococcosis is similar to that of other chronic lung infections, except that there are frequently less symptoms than the extent of the disease as revealed by physical signs or by radiography would indicate. Fever is usually mild, and often absent. Cough and sputum production are not prominent. Pleural pain is encountered occasionally and effusion rarely seen. Physical examination usually reveals dullness and diminished breath sounds, but rales and rhonchi are rare, because there is little exudation into the bronchial tree.

X-ray studies yield a wide variety of pictures, from that of mild bronchitis to large dense shadows with either distinctly or poorly defined borders suggesting neoplasm or abscess. There may be accentuation of the linear markings, surrounded by wooly shadows. Small nodules suggesting miliary tuberculosis or sarcoid may be seen, although in contrast to these disorders the bases of the lungs are more likely to be involved. Cavitation occurs only rarely. The mediastinum is usually spared, in con-

trast to other deep fungous diseases. The majority of cases exhibit bilateral involvement.

In some cases the pulmonary disease itself may increase sufficiently to cause death, but more often dissemination to the central nervous system or to other organs occurs. However, some cases of pulmonary cryptococcosis have apparently proceeded to recovery, leading Stoddard and Cutler, Sheppe, and others to speculate that there may be considerable tendency toward healing in the primary pulmonary form. Since cryptococcosis is thought to be rare, it is seldom seriously considered as a diagnosis in lung disease unless it is accompanied by central nervous system signs or symptoms, or those referable to other organs, and it is entirely possible that it actually occurs *frequently*, and heals without *recognition*, even as is known to be the case with some other deep mycoses (see coccidioidomycosis and histoplasmosis).

Disseminated Cryptococcosis

The remarkable predilection for involving the central nervous system is well known. As mentioned before, Semerak has suggested that the brain could become infected by direct spread of *Cryptococcus neoformans* from the nasopharynx, in which event the disease in this form could not be appropriately labelled "disseminated." However, this has never been conclusively demonstrated, and in the majority of instances a history of a preceding chronic pulmonary disease is obtainable

The symptoms and signs of invasion of the central nervous system are usually referable to increased intracranial pressure, less often to a meningitis (of less than the usual inflammatory nature). Headache usually appears early, intermittent and frontal at first, later becoming more severe, persistent and generalized Occasionally

the onset is sudden and violent, accompanied by projectile vomiting, suggesting subarachnoid hemorrhage. Dizziness, vertigo and nuchal rigidity soon appear. Eye signs often occur, including amblyopia, diplopia, mystagmus and ptosis. Periods of restlessness, disorentation, and hallucinations alternate with episodes of depression and loss of affect, sometimes simulating a definite psychosis, especially if the characteristic headache has not yet appeared. Epileptiform seizures occur occasionally.

Of special importance is the tendency for this disease to be accompanied by only a mild degree of inflammation, while one or more large space-consuming accumulations of diseased tissue develop. This picture can closely simulate neoplasm, and not infrequently the diagnosis of cryptococcosis has been made by surgery directed toward the removal of such "tumors" Since such focal lesions may occur anywhere in the central nervous system, the signs and symptoms will be extremely variable, and cannot be discussed here.

The cerebrospinal fluid frequently, although not invariably, furnishes evidence leading to the diagnosis. There is usually increased pressure; the fluid is discolored to a dirty yellow; the cell count is raised and predominantly lymphocytic, the protein is increased and the sugar diminished. A pellicle usually forms after standing for some hours, as with tuberculosis. The colloidal curve may be normal or of the meningitic type The characteristic singly-budding cells of *Cryptococcus neoformans* can often be seen by direct microscopic examination, especially if India ink is added to the specimen to provide the contrast necessary to see the capsules. The fungus is easily recovered and identified by culture on Sabouraud's medium at room temperature. Without such laboratory confirmation of its fungous nature, the entire picture can sim-

ulate mildly inflammatory tuberculous meningitis, and this diagnosis has undoubtedly been erroneously made on many occasions.

Although there may be short remissions, the disease progresses slowly to death, usually within a few months. No case has been recorded in which recovery ensued after *the diagnosis was definitely established.* However it is entirely possible, and even considered likely by some authors that cases of mild infection may occur and become healed without the disease being recognized.

There is associated enlargement of the lymph nodes, spleen and liver in about one-fifth of the cases of cryptococcosis, *frequently simulating Hodgkin's disease or one* of the other lymphoblastomas. This interesting and important point will be stressed later. Infection of the skin occurs only in about 5% of cases of cryptococcosis, most frequently as an eruption of the face consisting of translucent papules suggesting early vesicle formation, sometimes *simulating basal-cell carcinoma*, except that the border is sloping instead of abrupt. Although sometimes termed "acneform" these lesions do not become truly pustular, but instead their apices become necrotic and discharge a tenacious, translucent grayish or brownish-red material, consisting almost entirely of the encapsulated budding fungous cells with little or no contribution of purulent cells from the host. Such lesions as these may heal, and are not of such serious prognostic importance as central nervous system involvement.

Multiple, widely disseminated lesions in bone occur in somewhat less than ten per cent of cases according to Collins, producing osteoytic rarefactions, especially in bony prominences, and suggesting coccidioidomycosis or sarcoidosis rather than the proliferative changes of tuberculosis or actinomycosis. Lesions have also been reported

in kidneys, adrenals, pancreas, testes, bone-marrow and great blood vessels.

Pathology

Lesions of cryptococcosis are usually characterized by a startling mildness of the inflammatory response on the part of the host to the invading fungi; sometimes there is none at all, especially in brain involvement. On histopathologic study this is revealed by an almost complete absence of cellular infiltrate in or around the masses of fungous cells, which accordingly appear almost as though in pure culture. A thin mantle of lymphocytes may be seen at the periphery. These masses constitute the so called "gelatinous tumors", which by their enlargement exert enough pressure on the surrounding tissues to simply push them aside, a process earlier thought to be "histolytic" and thus responsible for a previous name for the fungus *(Torula histolytica)* Large gelatinous cysts of this type several centimeters in diameter are frequently found in the lungs. In the central nervous system the subarachnoid space is frequently found distended by similar gelatinous material, and cystic masses occur in the gray matter. The spinal meninges are often involved, but rarely the cord itself

Some cases exhibit a fibrotic reaction around the lesions, indicating that some healing can take place. In such cases there is a heavy chronic inflammatory lymphocytic infiltrate, in some areas granulomatous and tuberculoid.

Immunologic Aspects

In most cases of cryptococcosis it has been strikingly evident that the human body was making almost no effort to resist the disease either by immunologic mechanisms or by cellular infiltration. This fact, taken in conjunc-

tion with the knowledge that the causative fungi are encountered with great frequency by many persons makes it very difficult to account for the rarity of the infection. As with the other deep mycoses, it must be considered possible that the size of the original inoculum is the all-important feature. Certainly mice are uniformly killed by the usual laboratory procedure of intraperitoneal inoculations of *Cryptococcus neoformans*, whereas they do not apparently become fatally infected with any great frequency by whatever quantities of the same organisms they undoubtedly contact in their natural environment.

On the other hand, it may be that *normal* persons resist the fungus so completely as to never allow a lesion to result, leading us to conclude that only those individuals who are *abnormal* in some important phase of immunologic resistance can acquire the disease. It will be recalled that a similar theory is held with regard to coccidioidomycosis, in which it is believed that only those persons who possess an inherent defect in their immunologic mechanisms fail to fight the disease quickly and entirely successfully, and thus subsequently allow it to proceed to the serious, granulomatous, disseminated form.

As with histoplasmosis, there is a highly significant percentage of cryptococcosis patients who also show some disease of the reticulo-endothelial system, perhaps resulting in an immunologic defect.

It is, of course, possible that pulmonary cryptococcosis occurs in large numbers of persons in a form too mild to have been as yet recognized as such. Our knowledge of this same stage in coccidioidomycosis and histoplasmosis has been derived largely from studies utilizing their respective skin tests in large masses of "normal" persons; in cryptococcosis such skin tests have not as yet been carried out extensively, apparently because most workers

have concluded that the antigens so far obtained were impotent, having been unable to elicit positive reactions with any such material. The literature contains only a few scattered reports of positive reactions to intradermal injections in isolated cases (Berghausen, Kessel and Holtzwart).

Too little information is at hand to permit more than mere speculation, but it seems at least possible that the situation is analagous to that of the early workers with coccidioidin, who similarly decided that *that* skin test was valueless, since it gave negative reactions in most cases of proved serious coccidioidomycosis (see page 22). As was later learned, the test was always right, it was the observer's interpretation which was wrong, because patients almost always tend to become non-reactive to coccidioidin as a fatal termination approaches. Since virtually *all* cases of proved *cryptococcosis* proceed to death, why should we be critical of a specific test which yields only negative results when pitted again them? In fact, this is the only result which would be consistent with our experience in the other deep mycoses in which anergy to intracutaneously injected specific antigen is the *rule* in fatal cases. Thus the "torulin" testing, as heretofore carried out almost exclusively on proved cases of cryptococcosis, may have been performing its duty with great accuracy, instead of deserving to be classed as worthless The true status of this procedure cannot be known until large numbers of "normal" persons are tested with similar material.

The capsular substance of *Cryptococcus neoformans* is almost entirely polysaccharide in nature Evans and Mehl isolated and purified polysaccharides from three types of the fungus, which they designated as A, B and C Since the ability to serve as specific skin testing material in coccidioidomycosois, histoplasmosis, blastomycosis

tion with the knowledge that the causative fungi are encountered with great frequency by many persons makes it very difficult to account for the rarity of the infection. As with the other deep mycoses, it must be considered possible that the size of the original inoculum is the all-important feature. Certainly mice are uniformly killed by the usual laboratory procedure of intraperitoneal inoculations of *Cryptococcus neoformans*, whereas they do not apparently become fatally infected with any great frequency by whatever quantities of the same organisms they undoubtedly contact in their natural environment.

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prevent loss or denaturation of any potent material which may be present.

Agglutination reactions have received considerable study by Cox and Tolhurst, Benham, and Rappaport and Kaplan, using rabbits and guineapigs injected with killed and living *Cryptococcus neoformans* organisms. Reactivity in dilutions as high as 1-128 were encountered, but with such irregularity that the significance of the procedure must remain in doubt.

Cox and Tolhurst also attempted precipitin testing with animal cryptococcosis, and obtained some titers as high as 1-128. They drew no conclusions as to the significance of this reaction.

Treatment

There is no therapy which has proved effective for cryptococcosis beyond palliation. There are isolated reports of cases responding to one or another drug, but failures with the same drugs when used by other investigators probably indicate that remissions occurred instead of cures. Accessible abscesses or gelatinous cysts may respond to incision and evacuation or drainage. Amputation of an involved limb has occasionally prolonged life. Potassium iodide in large doses, and x-radiation have also been of assistance at times.

Since cultures of *Cryptococcus neoformans* do not survive temperatures above 105° F. to 107° F. for more than 7 days, several authors have suggested that artificial fever should be employed. As yet there has not been enough clinical trial of this modality to furnish any conclusive evidence of its value. Against this view is the fact that rabbits, which have a high normal temperature succumb easily to the infection, and that in general, febrile patients die more rapidly than afebrile ones. Mosberg,

and sporotrichosis resides apparently in the polysaccharide portion of the material derived from the corresponding fungi, perhaps these "torulins" of Evans and Mehl may prove to be ideal for intradermal testing in cryptococcosis. However, there is some evidence derived from animal experimentation which casts doubt on this idea. Kligman failed to demonstrate any reactivity to intradermal testing in infected rabbits or mice. This might *still* be explainable if these animals were *fatally* involved, and hence in the anergic stage.

Several authors have reported inability to obtain any fixation of complement using serum from patients possessing cryptococcosis and antigens derived from *Cryptococcus neoformans.* This is a notable departure from the results in fatal cases of the other deep mycoses in which this reaction usually occurs in high titer. It has generally been simply concluded that *Cryptococcus neoformans* is not able to *stimulate* such antibody production in the host because of the comparative isolation provided by its capsule. However, it seems at least equally plausible that the fault may lie in the *antigen* which is used in the *test.* It will be recalled that in other deep mycoses effective complement fixing antigens apparently must contain some nitrogen, probably in protein-like groupings, and that this component is easily destroyed in extraction and sterilization procedures. Since the majority of the mass of *Cryptococcus neoformans* as obtained from cultures consists of capsular material which is almost entirely polysaccharide, it is probable that special techniques will have to be developed to remove the excess capsular polysaccharide or to obtain extracts from the centrally located fungus cells themselves, before success can be obtained. Such extraction should be very gently accomplished to

Chapter 18

SOUTH AMERICAN BLASTOMYCOSIS (PARACOCCIDIOIDO-MYCOSIS)

THE TERM "South American" is appropriate for this disease, since it is limited entirely to that Continent, but it is even more sharply confined to certain smaller regions within that area. In spite of this, it should not be given any less of our attention than the other deep mycoses, first, because it kills at least as many persons, and second, because it presents many features which differ from the others and which if fully understood might furnish valuable clues to new approaches for study. That the subject is not easy was impressed upon this author upon observing with what extreme diligence research is being carried out by highly intelligent personnel in several cities in Brazil, without as yet having achieved complete success It must be emphasized, however, that it was the last of the seven deep mycotic disorders to be discovered and its investigators should therefore logically be permitted to have some extra years for study to make up for such a late start.

In 1908, Lutz, in São Paulo, Brazil observed two patients who presented lesions in the mouth accompanied by cervical lymphadenopathy He isolated the causative organism, identified it as a fungus, described its method of multiplication by a budding process, and named the disease "pseudococcidioidal granuloma." Carini and Splen-

Alvarez and De Chondens advocated combining fever therapy with "alkalinization."

Until more is known, the recommendations made for other deep mycoses should be followed, namely, a high caloric, high protein diet, with supplemental vitamins, especially of the B series, bedrest, and the avoidance of steroid hormones and wide spectrum antibiotics (see pages 75 and 76).

The point of ingress is almost always somewhere in the gastrointestinal tract, most commonly the mouth, but in a significant percentage of instances the tonsils, larynx, nose, intestine or anus. Occasionally the eye furnishes the portal of entry. From any of these primary foci the infection becomes disseminated by both the lymphatic and hematogenous routes, and may eventually affect practically every organ of the body.

Azulay pointed out that this disease seldom remains sufficiently limited to any one organ system to allow it to be classified into various types by such features. He recommended separation of the cases into "tegumentary" and "extra-tegumentary" forms. His term "tegumentary" includes mucosal as well as cutaneous lesions.

Tegumentary Form

The primary lesions, usually occurring on the mucosal surface of the gums, cheeks, lips, palate, tongue or nose, are ulcerated and reddened areas, exhibiting tiny yellowish spots, called by Pupo "mulberry-like erosive stomatitis." In continuity with areas of mucosal involvement, cutaneous lesions occur by direct spread. The skin also becomes involved secondarily by the ulceration and fistulization of infected lymph nodes in a manner simulating scrofuloderma. In addition, skin lesions occur by hematogenous dissemination, and may be papular, pustular, tuberous, ulcerated or vegetative and verrucous. The commonest site is the face, but the trunk and extremities are frequently involved as well.

An unusual form of cutaneous involvement has occasionally been observed, consisting of keloidal areas usually situated on the back and legs First described by Jorge Lobo, this may be simply a variant of South American blastomycosis, or it may be a different disease caused by another organism.

dore soon confirmed his findings, and many papers subsequently appeared, some of which indicated that the disorder was being confused with coccidioidomycosis. In 1929 Almeida clarified this issue with a series of reports of careful studies concerning mycology, pathology and symptomatology. Bogliolo and Aroeira Neves, Lacaz and Fava Netto, and Aleixo and Furtado have more recently contributed much information.

South American blastomycosis is encountered principally in the Brazilian States of São Paulo, Rio de Janeiro and Minas Gerais, and in Argentina. The incidence is highest in the ages from 20 to 50, and males outnumber females 6 to 1. Although the habitat of the causative organism in nature has not yet been established, the clinical aspects of the disease indicate strongly that the portal of entry is usually the oral mucosa, and the prevalence of the infection among agricultural workers who habitually clean the teeth with bits of vegetation, and who chew the stems and leaves of various plants, seems to mean that the organism lives on vegetation or in the soil. Direct transmission from man to man, or from animals to man has not been proved.

Clinical Characteristics

On the basis of present knowledge it is not possible to divide South American blastomycosis into the types utilized for the previously discussed deep mycoses. Primary inoculation into the skin may occur, but convincing evidence is difficult to obtain in most instances.* Although the lungs are involved in a high percentage of cases, they are only rarely accepted as the primary portal of entry.

*The author observed a case of a laboratory worker who inoculated a finger with a viable culture of B brasiliensis. Biopsy revealed the organisms, but there was no subsequent evidence of infection

The fungus is most easily found in giant cells or microabscesses, and occurs as singly-budding or multiply budding cells from 6 to 60 microns in diameter. Cultures and animal inoculation (mice or guinea pigs) frequently must be resorted to before the diagnosis can be established.

Immunology

As has been previously mentioned little is as yet known about the immunologic aspects of South American blastomycosis. In the majority of diagnosed cases there seems to be little evidence of immunologic resistance on the part of the host. It is, of course, entirely possible that the infection is acquired by a much larger number of persons, and is resisted so well that it remains entirely subclinical throughout its course to complete recovery, as is known to be the fact in coccidioidomycosis and histoplasmosis, and postulated from a fair amount of evidence in some other mycotic infections. However, such a mild form has not as yet been shown to exist, and judgment must be withheld until mass intracutaneous testing of the "normal" population with potent antigens can be carried out. Until recently the intradermal injection of antigens prepared from cultures of *B. brasiliensis* have not yielded uniform results in the hands of different investigators, but Almeida, Lacaz and Cunha consider the test of value both in diagnosis and in prognosis.

As far as it has been studied, the intracutaneous reaction is in no way inconsistent with experience with coccidiodin Severe, fatal infections produce anergy to the test, while milder ones yield varying degrees of reactivity Recently Lacaz and Fava Netto have isolated a polysaccharide, purified by several methods, which may prove to be more specific than previously available materials

Extra-tegumentary Form

The lymphnodes are involved sooner or later in most cases of *South American blastomycosis, especially those* of the neck, which drain the usual site of primary inoculation. Sometimes the primary lesion is not visible, and swollen glands are the only presenting sign. Often there is enough generalized lymphadenopathy to simulate Hodgkin's disease. Involved nodes tend to ulcerate and drain through the skin.

The lungs are affected in a high percentage of instances, up to 94% in the opinion of some authors, but this fact has only recently become evident. Pulmonary infection exhibits a variable picture, but in general the *signs and symptoms tend to be milder than* would be expected from the degree of involvement revealed by x-ray studies or at autopsy, which may include large and small nodules, miliary dissemination, fibrosis, small cavities and thickened pleurae. Lesions tend to be bilateral, and prefer the bases of the lungs, or seem to spread from the hilar regions while leaving them relatively free.

The lower gastrointestinal tract seems to be the primary inoculation site at times. Appendiceal lesions occur, and involvement of the ano-rectal area is not rare. The spleen and liver may be enlarged.

Pathology

There is nothing specific about the macroscopic and microscopic changes in the tissues, but they more closely resemble those of North American blastomycosis than those of any other entity. Histopathologically, granulomatous areas, either of the tuberculoid or foreign body types are seen, but it is necessary to find and identify the causative organism before the diagnosis can be made.

periods of time. Clinical improvement is obtained in most cases, some of which apparently become cured. However relapses are frequent upon discontinuance of the drug, and toxic manifestations make such long-term administration difficult. Various combinations of sulfonamides are utilized. As with the other deep mycoses a diet high in protein content, and supplemental vitamins of the B group and C, should be maintained.

Lacaz, Almeida and Cunha believe that hypersensitivity to this test indicates ability to resist the infection.

The complement fixation reaction has not as yet been very extensively studied, but here again, the observations so far seem to parallel those in coccidioidomycosis. In almost 200 cases of severe type tested by Lacaz and Fava Netto, complement fixation was demonstrated in all but two, one of which may have been tested too early in the course of the disease for this reactivity to have developed (These, of course, were all seriously involved cases, similar to those in which we would expect complement fixation to occur uniformly if coccidioidomycosis were the diagnosis.) There are indications that a high titer indicates a poor prognosis, and that recovery may be heralded by a decreasing titer as is the case in coccidioidomycosis and probably in other deep mycoses (Lacaz and Fava Netto).

The titer of complement fixation has not been nearly as high as in coccidioidomycosis or histoplasmosis, resembling more closely North American blastomycosis in this regard. This may be due to too much "purification" of the antigen, or to heating. For example, the "pure poly-saccharide" of Lacaz and Faxa Netto is probably not the *ideal* antigen for this reaction; a similar preparation made from coccidioidin fails to fix complement *at all.* Better material for preliminary studies would probably be obtained by sonic disruption of the living cells, and simple filtration. When more is learned, attempts to purify and store this antigen by methods which conserve any *protein*-like components which may be present would be advisable.

Treatment

Sulfadiazine is the most extensively used drug, administered in a dosage of from 2 to 4 gm. daily over long

disorder was soon emphasized by Ponfick. The discovery of tiny clumps of the causative fungi in pus and in tissue sections lead Harz to name the organism *Actinomyces* ("the ray fungus"). After prolonged and careful study, Wolff and Israel cultured this fungus in 1891.

Actinomycosis results most frequently from infection by *Actinomyces bovis*, the only pathogenic fungus which prefers to grow anaerobically. It is now usually agreed that there is only this single species within this genus although some authors still believe that human infections are caused by a slightly different species, called *A. israeli*. A considerable percentage of cases is caused by several fungal species which are closely allied morphologically but differ by growing only aerobically and are classed in the genus *Nocardia*, first reported differentially in 1896.

Actinomycosis has been observed in all parts of the world, indicating either that man carries the causative organisms with him in a saprophytic fashion wherever he goes or that they are universally distributed in nature. Both of these mechanisms are operative in the etiology of actinomycosis *A bovis* has been isolated from normal mouths and throats, and can be considered to be probably usually present in these locations and carried from them into the gastro-intestinal tract of most healthy human beings and animals. Emmons found it in 47% of cases in the crypts of surgically removed tonsils, and obtained cultures in half of them Rosebury found it in carious teeth and in the calcareous deposits around extracted abscessed teeth.

When an organism such as this which ordinarily exists as a harmless saprophyte, succeeds in causing disease, the infection is said to be endogenously acquired, other more familiar examples are those due to certain streptococci and staphylococci It is extremely important to

Chapter 19

ACTINOMYCOSIS (LUMPY JAW) (INCLUDING NOCARDIOSIS AND MADUROMYCOSIS)

AMONG THE members of three genera within a large group of primitive bacteria-like fungi known as the *Actinomycetes,* there are a few species which are pathogenic for man and animals. Some of these cause a chronic granulomatous and suppurative disease, which, while exhibiting considerable variation in its clinical course, can nevertheless be roughly considered as a definite syndrome. It is characterized by intense inflammatory induration, accompanied by the development of deep abscesses, which eventually rupture, leaving persistent draining sinuses Certain other fungi, not even remotely related to those mentioned above, are capable of duplicating this clinical picture. While not beyond criticism, this syndrome will here be called actinomycosis, regardless of the specific causative organism. Of all the so-called "deep mycoses" it is the most commonly encountered.

Actinomycosis was the first deep mycosis to be delineated, as a disease of cattle called "lumpy jaw," by Bollinger in 1877.* In the same year Israel reported a disease in man, the similarity of which with Bollinger's

*It is interesting to recall that Koch's discovery of the tubercule bacillus did not occur until 1882

not carried endogenously by man another explanation must be sought. There is probably sufficient reason in the fact that they are found in soil from all parts of the world. As might be surmised, infections of this type usually begin in the lungs where the organisms have been carried by inhalation with dust, or in the feet because of direct inoculation of soil into wounds Nevertheless it is necessary here also to emphasize the importance of associated trauma or bacterial alliance in conferring pathogenicity only occasionally on organisms so frequently encountered, otherwise the disease would occur much more often than is the case. Little is known of the epidemiology of the disease when caused by fungi other than *A. bovis* or *Nocardiae*, but it is probably very similar to the latter.

Actinomycosis can occur at any age, but predominates in male adults, probably because they are more subject to the appropriate forms of trauma and maintain a poorer grade of hygiene in the mouth and extremities

Clinical Characteristics

There are several well defined clinical forms of actinomycosis. In considerably more than half of the cases, it occurs in the region of the face or neck and is called the "cervico-facial" type. The abdomen is the site in about twenty per cent and the chest in fifteen per cent In the small remaining percentage of instances the area elected is extremely variable, in the approximate order f frequency the involvement occurs in the extremities, e skin, bones, joints, kidneys, ovaries, liver or central rvous system However, it is often evident that such ons have arisen by dissemination from one of the three re common types

consider the contributory factors which endow such ordinarily innocuous organisms with pathogenic powers, because measures directed against such factors may be more effective in prevention as well as in therapy than those antagonistic to the microbes themselves. In actinomycosis due to *A. bovis,* the most important of these factors is trauma, especially that which presents the fungus with injured devitalized tissue far enough from the surface of the body to furnish the anaerobicity which it desires. Thus actinomycosis frequently follows the extraction of infected teeth, fractures of the jaws, and bites inflicted by the teeth of animals or human beings. The presence of disease due to other causes may also serve by lowering resistance. Another important factor, especially where therapy is concerned is that *A. bovis* seems to establish a symbiotic alliance with certain endogenous bacteria, the combination exhibiting a degree of pathogenicity not possessed by either ally alone. As a consequence, some of the effectiveness of sulfonamide and antibiotic therapy in actinomycosis is probably due to antagonism against bacterial allies rather than against the fungus itself. It is significant in this regard that usually it has been impossible to infect animals experimentally with *A. bovis* in the absence of trauma and concomitant bacterial infection. These facts seem adequate to explain the universal distribution of actinomycosis, since wherever man exists he carries with him these organisms awaiting only the proper opportunity to cause disease. It was previously taught that an important factor was the habit of chewing grass or straw by agricultural workers, but the fungi have not been found on such materials, and a more likely explanation is contained in the above thesis.

However, clinical actinomycosis caused by *Nocardiae* is also universally observed, and since these organisms are

The bone is often shown to be involved by x-ray studies usually beginning as a periostitis indicating that the infection approached the bone from the adjacent tissues instead of being blood-borne. Osteomyelitis develops, followed by areas of destruction and rarefaction combined with bony proliferation, producing fuzzy, irregular borders. Occasionally, however, smoothly outlined cysts are seen, indicating hematogenous dissemination.

Abdominal Actinomycosis

Since the causative fungi are frequently present within the intestine in normal persons, injuries puncturing its wall can initiate abdominal actinomycosis. Frequently, an apparently nontraumatic form originates in the appendiceal area. Sometimes the ovarian tubes, gall bladder or liver seem to be the primary focus. Sometimes a mass can be palpated. Differentiation from other types of appendicitis, salpingitis, cholecystitis, cystitis or pyelonephritis is very difficult. The correct diagnosis is usually made only after exploratory laparotomy, unless some of the abscesses previously have approached the surface of the body and ruptured to produce the typical draining sinurses from which the organisms can be recovered. X-ray studies may help by revealing suggestive areas of involvement of the proper type in the bones of the pelvis or vertebrae. In contrast to tuberculosis, actinomycosis favors the articular facets, laminae and the transverse and spinous processes rather than the vertebral bodies, according to Simpson and McIntosh.

Thoracic Actinomycosis

Primary pulmonary actinomycosis is caused by the aspiration of material from the mouth, or by inhalation of dust. It is impossible in the early stages to differen-

Cervico-facial Actinomycosis

As has been previously pointed out, the portal of entry for *Actinomyces bovis* is most frequently the region about the teeth, gums, jawbone or the tonsils. The infection frequently follows the extraction of infected teeth or fractures through the tooth-bearing areas of persons maintaining poor oral hygiene. The mandible is most often involved, usually near the angle of the jaw. The region of the maxillary sinus is also particularly susceptible. Primary infections have been described in the lacrimal glands, the orbit, the tongue, or in the lower pharynx or larynx.

At first the infection does not differ from the more common bacterial infections of low grade, but soon the overlying skin becomes darkly purple or red and beneath it a degree of induration develops of such firmness as to have been called "woody" (ligneous phlegmon). There is often much limitation of motion and muscular spasm. Slowly, the surface becomes irregularly swollen, abscesses develop periodically and finally rupture or are incised, leaving thereafter sinus tracts which persist for months, discharging serosanguinous or purulent fluid in which tiny yellowish-white friable masses of the causative fungi can be seen, the so-called "sulfur granules." Healing of some areas occurs by firm cicatrization while others nearby are developing into new lesions. All stages of the above processes are usually present simultaneously in contiguous areas. The disease exhibits but little tendency toward complete clearing, and often continues chronically for years. The symptoms are usually less than the extent of the disease would indicate, and as long as it remains well localized the general health of the patient is but little affected.

which is caused by the location in the body, since from the clinical standpoint the basic picture is the same.

Pathology

Grossly, the pathology of actinomycosis is characterized by a dense cellular infiltration producing in general a surprising firmness, but becoming softened in many areas by abscess production, leading to sinus formation which is then gradually followed by cicatricial healing in most instances. All of these processes can be seen in contiguous areas simultaneously. The infection spreads by burrowing along fascial planes, leaving much intercommunication among the residual sinus tracts. Cavities filled with purulent and necrotic debris are common.

The typical microscopic picture is that of an abscess in which actinomycotic granules up to 25 microns in diameter may be seen in the appropriately cut sections. The central portion is surrounded by polymorphonuclears Next is a zone of granulation tissue containing histiocytes filled with lipoids Leucocytes, plasma cells and connective tissue cells form the abscess wall. When stained with hematoxylin and eosin the granule of *A. bovis* exhibits a central basophilic area, irregularly circular in outline, which can sometimes be seen to be composed of closely packed, branching tiny filaments about one micron in diameter. This dark area gradually shades into an acidophilic peripheral zone in which filaments are distributed as though they were radiating from a central focus. Many of these filaments seem to be enlarged at their tips by being surrounded by a gelatinous sheath, forming "clubs," now thought to be probably contributed by the host as a part of a defense process. In *Nocardia* infections actual granules may not be present, especially when *N asteroides* is the causative agent, and only groups of tiny branching

tiate it from other low grade chronic pulmonary diseases. The early appearance of expectorated bloody pus indicating the presence of small abscesses is helpful. Sometimes the process can penetrate through the chest wall and present at the surface of the body the typical discharging sinuses of actinomycosis, even without signs of the pleura having been involved. The symptoms include dyspnea, fever, night sweats, anemia and progressive wasting. Physical signs are sometimes of value in differentiating from tuberculosis by revealing the bases rather than the apices to be involved. X-rays usually also reveal basilar involvement, more commonly bilateral, consisting of consolidations extending outward from the hilum, simulating neoplasm. Sometimes the differentiation can be made by observing small areas of rarefaction within such masses, caused by actinomycotic abscesses. Pleural effusion is frequently seen, and adhesions are common. Invasion of the ribs often occurs, and conforms to the type of bone involvement previously described.

Other Forms of Actinomycosis

Any part of the body may become involved with actinomycosis either by direct extension or hematogenous dissemination, and a wide variety of symptoms and signs be thus produced. Carrion and Cope have covered this subject more thoroughly than is appropriate here.

Infection occurs by direct inoculation through or into the skin, most commonly of the foot or leg. Ulceration develops, which gradually penetrates deeper and eventually assumes the classical picture of actinomycosis. It has been the custom for most authors to treat this form separately from actinomycosis, under the headings of "Madura foot," "maduromycosis" or "mycetoma." It seems, however, that the principal difference is only that

In addition to the organisms previously mentioned a wide variety of fungi have been implicated in actinomycosis, particularly in involvement of the feet (maduromycosis, mycetoma) sometimes under circumstances casting considerable doubt upon the accuracy of the identification of the causative agent, and often depending upon a single reported case. This extremely complicated subject cannot be resolved here, and the reader is referred to an extensive literature for what clarification is required in rare specific instances.

Of all of these, the most firmly established as a cause of madura foot is *Monosporium apiospermum*, the perfect stage of which is called *Allescheria boydii*. In this case the hyphae are much larger than the actinomycetes, and spores are sometimes seen at the periphery of the granules.

Immunology

Although there have been many reports concerning the allergic and immunologic aspects of actinomycosis, it is not as yet possible to correlate the reactions in any constructive way with the clinical course of the disease, or to use them reliably for prognosis in the manner so helpful in some other deep mycoses Some of the inconsistencies in these studies are doubtless due to variations in the antigens employed, both because of extensive differences in the chemical and physical methods by which they have been prepared as well as the fact that there are several species of fungi involved among which it is probably not correct to assume identical non-specific cross reactivities. *Actinomyces bovis*, for example, is so difficult to culture that it tends to die out after a transfer or two, which indicates that it is perhaps not sufficiently well pleased by such artificial environment to produce antigenic sub-

filaments will be seen taking the basophilic stain, and exhibiting a moderate degree of acid-fastness. These elements are somewhat easily confused with tubercle bacilli.

Diagnosis

Actinomycosis usually presents a strongly suggestive clinical picture, rather easily confirmed by biopsy and histopathologic study or by culture. *A. bovis* will be missed entirely in cultural studies unless anaerobic media are included, while the *Nocardia* species require aerobic media. Granules furnish the best inoculum when present, and can be more easily seen if the exudate is allowed to flow down the side of a test tube held against the light or to soak into a gauze pad or dressing. It is not correct, however, to assume that the discovery of granules identifies the disease as actinomycosis, since similar granules are occasionally produced by bacteria of several types (actinobacilli) or may be composed of coagulated fibrinous material. It is also wrong to *exclude* actinomycosis because of the *absence* of granules, since some organisms do not form them (*N. asteroides*). It is therefore necessary to identify the fungus, by microscopic examination at least and by culture whenever possible.

In maduromycosis the granules may be differently colored, most commonly black or reddish. These variations are apparently not consistently attributable to specific fungi and cannot be relied upon for identification of the organism involved.

There are at least four species of *Nocardia*. *N. asteroides*, *N. madurae*, *N. brasiliensis*, and *N. paraguayensis* (Ochoa). Some authors further divide these into additional species. Some of these organisms may belong to the genus *Streptomyces*, particularly the last named.

duced" and "reduced" by absorption of other strains of *Streptomyces* which are always present as soil fungi in large numbers in the intestinal tract of all persons.

In contrast to this view, it must be emphasized that in some other deep fungous infections, the development of hypersensitivity is the rule rather than the exception, and that the greater the degree of allergic reactivity of the delayed tuberculin type demonstrated by the patient's skin, the greater is the likelihood of complete recovery from the disease through the development of specific immunologic resistance. With regard to actinomycosis the work of Gonzalez-Ochoa is especially significant in this light. He has prepared a "purified polysaccharide" derivative from *Nocardia brasiliensis* which he found to be highly specific in persons bearing infections due to this organism. In a series of such cases, he found hypersensitivity of the delayed tuberculin type to be revealed by intracutaneous testing in all patients who resisted the infection well enough to become cured eventually, while three who succumbed were consistently non-reactive. This parallels experience with the prognostic significance of the coccidioidin skin (thought also to be almost if not entirely due to a polysaccharide) and conforms to what seems to be a trend in histoplasmosis and the blastomycoses; in all of which diseases hypersensitivity of this type appears to be related to the patient's ability to resist the progress of the infection immunologically In this light, artificial desensitization would appear to be illogical and perhaps even dangerous. At this time it seems warranted to advise against placing reliance in such "vaccine" therapy, or indeed before considering it as entirely safe

There are several reports concerning studies of complement-fixing antibodies as well as agglutins and precipitins found to be contained in the sera of persons infected

stances at all comparable to those connected with its pathogenic activities in the infected body.

It also seems probable that such testing procedures have often been classed as useless because they did not yield results consistent with the *preconceived theories* of their observers. As has been shown, particularly with coccidioidomycosis, such tests should be allowed to speak for themselves and *our* theories adapted to *conform* to their conclusions wherever possible.

The intracutaneous injection of culture filtrates derived from organisms in this group have yielded both local and systemic reactions of hypersensitivity in persons possessing actinomycosis. There have been various interpretations of these reactions. Mathieson and co-workers concluded that some persons finally become hypersensitive allergically to *A. bovis* by repeated absorption of the fungus or its products from its saprophytic habitat in the normal body, and that they thereby become susceptible to its pathogenic invasion. They advance this concept to explain the comparative infrequency of the actual infection by organisms so often present in normal individuals. In such a circumstance, repeated injections of extracts has been recommended as producing a degree of "desensitization" of value in increasing resistance to the infection, and even inducing specific immunologic resistance. In this regard it is interesting to speculate on the possibility that some of the beneficial influence exerted by antibiotics upon the course of actinomycosis may be due to such a desensitization mechanism, since most of these substances are derived from fungi of the genus *Streptomyces*, which are so closely allied with *Actinomyces* and *Nocardia* that they might well produce antigens of closely similar nature. It is difficult to believe very strongly however that this same type of hypersensitivity would not be similarly "pro-

has been previously alluded to as one of the principal reasons for its acquisition of pathogenicity. It is true that in vitro certain strains of *Actinomyces bovis* seem susceptible to one or another of these drugs, but it must be recalled that cultures of this organism are not easily obtained nor maintained in vitro even *without* such antagonistic substances, making it difficult to assess their value. Selection of medicaments by such "specific testing" is permissable, but it is even more important to investigate in the same manner the bacterial flora which is also present and to select and administer the appropriate drugs to combat this factor as well. Nocardiosis apparently responds better to sulfonamides than to antibiotics, the reverse characterizes *Actinomyces bovis* infections. Apparently, neither form of chemotherapy helps the other forms of maduromycosis appreciably. Isoniazid has been recommended for *A. bovis* infections by McVay and Sprunt. Gonzalez Ochoa recommends 4, 4-diamino diphenyl sulfone for disease caused by *N. brasiliensis.*

Vaccino-therapy has been advocated by several workers, but it appears that this subject needs further cautious investigation in the light of the view that such procedures may actually be harmful The procedure known as specific desensitization is equally dubious.

Surgery should be postponed until after the initial amelioration is obtained by "specific drugs"; then surgical drainage should be adequate, and all tissue manifestly beyond recovery should be debrided. Intelligent use of antibiotics permits the surgeon more latitude in his procedures than was the rule before their advent

In the treatment of the chronic, low grade disease which remains after the above procedures have been carried out, drugs seem of little value Iodides may be used in maximum dosage, either orally if tolerated, or intra-

with actinomycosis, but there is not as yet enough consistency in the results to warrant the use of such procedures for diagnostic or prognostic purposes. As has been discussed in other chapters, perhaps an antigen which is not so highly "purified" as a polysaccharide, but instead *treated gently enough to retain its protein-like elements* in an "undenatured" manner, would yield more significant and reliable reactions.

It is worth emphasizing again that it also is possible that these discrepancies are largely due to the tendency on the part of the observer to consider a reaction worthless unless it conforms to his own theories as to significance. Perhaps these reactions actually should be interpreted as they are in coccidioidomycosis, in which disease they appeared to be equally irregular until better interpretation showed them to be consistently valuable.

Therapy

The advent of the sulfonamides and the antibiotics has indisputably bettered the prognosis of actinomycosis *to a marked degree, but none of these alone nor any combination of them should be trusted as specifically curative without adjunctive therapy.* Initially a dramatic degree of improvement follows such treatment, but soon a point is reached where further progress is extremely slow, even though adequate dosage is continued. It is true that the discontinuance of such medication is frequently followed by exacerbation and its readministration by a resumption of improvement, which seems to indicate that it is capable of holding the activities of the fungus in check. *It seems* more reasonable, however, that the majority, if not all of *the effect of such substances, should be attributed to their* ability to combat the concomitant bacterial infection and thus deprive the fungus of the symbiotic alliance which

venously. Heavily filtered x-radiation or radium implantation is of great value, especially in the cervico-facial type. In maduromycosis, amputation must still be employed in many cases.

It is extremely important to remember that the factor responsible for the ultimate *complete* eradication of the disease is almost certainly the body's natural ability to resist and heal the infection, whether this be by the development of specific immunity, phagocytosis, cicatrization or some other mechanism. Prolonged rest in bed with adequate nutrition and multiple vitamin supplements especially of the B complex series are vital.

Chapter 20

DERMATOPHYTOSIS—THE CLINICAL SYNDROMES

IN CONTRAST to the fungi heretofore discussed, there is a large group of organisms which limit their pathogenic activities to the skin and its appendages, and are hence called dermatophytes. Their inability to survive in any of the deeper tissues of the body usually has been accepted as well established. In fact, in all but a small percentage of instances their range is even narrower, they luxuriate in the *keratinized* portions of the *epidermis* and the hair and nails; they thrive less well in the rete and do not ordinarily penetrate deeper than the basal cell layer Thus they might be even more appropriately termed "epidermatophytes," or "keratophytes"

It is probably correct to state that the majority of human beings become infected with one or more of these organisms at some time in their lives, but in the vast majority of cases either in a subclinical form, or one so mild as to constitute only a minor nuisance. There is, however, a wide range of severity beyond this point, so that occasionally death may result, especially if there are concomitant bacterial infections or allergic phenomena.

It is interesting to note that as early as 1841 David Gruby had conclusively proved for the first time that a microbe could cause human disease by studying one of the skin mycoses of this type called "favus," culturing the

causative fungus from infected sites, reinoculating such cultures and reproducing the disease again, thereby fulfilling what some 40 years later became known famously as "*Koch's postulates*" in relationship to tuberculosis. Thus mycology is the oldest of microbial sciences.

Because of too much attention to minutiae, and a great deal of incomplete and inaccurate observation and reporting, during the ensuing century several hundred "species" of fungi were described and "named" as pathogens for the human skin. This complexity prevented the development of any satisfactory classification of diseases based upon the *causative organisms*. It was easy, however, to devise a useful system of classification dependent upon the clinical variations exhibited, which were in turn determined largely by the regions of the body infected, and this system has become universally adopted. Thus, an infection with one of these organisms is called a "dermatophytosis," or more commonly a "tinea" or "ringworm." Adding adjectives indicating the areas of the body which are infected results in the clinical classification still used today. In the last few decades competent mycologists have reduced the number of "species" of pathogenic fungi to but a few, and almost uniformly these have fallen into an admirably close relationship to this clinical classification

Tinea Capitis

Ringworm of the scalp exists in several different clinical forms, each one of which is rather consistently, although not invariably, caused by its own specific species of dermatophyte. While none of these is strictly limited geographically, there are tremendous percentage variations in their distribution over the globe.

The vast majority of cases occur in prepubertal children concentrated in the age groups from 5 to 11 years.

and predominating in males. Within this group there are two types, roughly differentiated as "noninflammatory" and "inflammatory." Except for odd percentage distributions encountered in certain constant geographic areas, and for chronologic variations due to epidemic manifestations these two can be called the "common" forms.

The common childhood "*noninflammatory*" type is usually caused by *Microsporum audouini*, and is always acquired by direct transmission from child to child, or indirectly by means of inanimate objects such as caps or theater seats, since animals cannot become infected with this organism. This infection has often reached epidemic proportions, lasting for years in some localities.

It begins as a simple scaling in one or more areas of the scalp, most commonly in the nape, which spreads from the point of inoculation peripherally to form round or oval patches, and eventually polycyclic areas by coalescence. Soon the hairs in the central portions of these areas become lusterless, and so brittle that they break off close to their point of emergence from the follicles. There is little tendency however for these hairs to become loosened in the follicles, so that they do not ordinarily become shed completely. There is usually but little itching, and the condition may easily remain unnoticed until it has become extensive. This form is extremely resistant to treatment, but when such areas finally do heal there is no permanent hair loss or scarring

The common, childhood *inflammatory* type of tinea capitis is characteristically caused by *Microsporum canis* (*lanosum* and *felinum* are synonymous). These infections are almost invariably acquired by a child through direct contact with an infected animal pet, most commonly a cat or dog. Seldom is direct transmission from child to child clearly evident, hence true epidemics do not occur.

Occasionally, similar cases will be found to be caused by *Microsporum fulvum (gypseum)* which is apparently contracted directly from soil where this organism grows saprophytically.

The initial lesion is probably a scaly condition in the scalp, but the disease is seldom noticed in this stage. An erythematous papular area is usually first seen, with hairs in the centers of the papules. Soon a patch is formed, within which the hairs lose their luster, become brittle and are broken off close to their bases. Later, the inflammation causes some hairs to become loosened in their follicles so that they fall out. Itching is usually present and may be severe. There may be but a single such patch; but, more often, extensive involvement eventually occurs.

All degrees of inflammation may accompany this process, but in each case all lesions tend to be in the same stage at a particular time. At first the inflammation is mild and may increase only gradually for a time, but in many cases it very suddenly becomes severe. At its worst, there is pronounced swelling, developing into deep, boggy ulcerative areas exuding pus, known as "Kerion Celsi." There may be severe constitutional reactions at this stage. Considerable scarring and permanent hair loss is to be expected after such areas heal.

In both the non-inflammatory as well as the inflammatory types, the diagnosis must be confirmed by microscopic examination of an extracted hair root prepared with KOH solution; a mantle of round to oval spores about 2 microns in diameter is seen to extend around the exterior of the shaft, occasionally accompanied by a hyphal strand. This distribution is called "ectothrix." Cultures are necessary, however, to differentiate the types accurately, since the degree of inflammation is too variable to be reliable.

Filtered ultraviolet radiation is a helpful adjunct to diagnosis, since the hairs involved in *M. canis* or *M. audouini* infections fluoresce a brilliant greenish yellow. There are some pitfalls, however, in this examination since fluorescence may not begin at once in the course of the infection; it may be destroyed by medication before cure has been attained, and is actually almost always absent entirely in *M. fulvum* infections. Also other substances such as keratin, soap, medicaments and dyes fluoresce in a disturbing manner.

The *third type* of tinea capitis is called *black dot* ringworm although this characteristic is by no means always present. It is transferred from human to human and hence it often exists in epidemic proportions. This form is caused by either *Trichophyton tonsurans* (synonyms, *sulfureum, crateriforme*) or *Trichophyton violaceum*. The former predominates in the Americas, the latter in Europe, Africa and Asia. It involves adults as well as children but less frequently. Clinically it can be relatively noninflammatory at times and resemble seborrheic dermatitis, or it can develop kerion-like inflammation resulting in scarring and permanent alopecia. The hairs tend to break off within the follicular orifice, where a blackened plug is left, from which characteristic the disease acquired its nickname. For unknown reasons, the placques in this disorder tend toward angulated perimeters, frequently forming polygons instead of ovals or circles. Unless marked inflammation occurs, this type is usually resistant to treatment.

Microscopic examination reveals the hair shafts to be invaded longitudinally by hyphal threads which tend to break up into box-like or subspherical "arthrospores," an arrangement known as "endothrix" involvement. Cul-

tures are necessary to establish this type of infection and to guide the prognosis. There is no usuable degree of fluorescence under ultraviolet radiation.

A fourth type of *tinea capitis* is occasionally encountered and caused by *Trichophyton mentagrophytes (gypseum)* or *Trichophyton verrucosum (faviforme).* The source is often shown to be infected animals, most frequently cattle, horses, rabbits, or rats or mice. Clinically, this infection tends to involve only one or a very few small areas, and to be markedly inflammatory rather early in its course. Kerion is common and frequently severe. The fungi are seen microscopically to be present in the ectothrix pattern, and the rounded spores to be somewhat larger than in *Microsporum* infections.

The last type of tinea capitis is called "*Javus*" and is caused by *Trichophyton schoenleini.* This is evidently transferred from human to human. Although there is considerable inflammation, the disease is extremely resistant to therapy and frequently lasts throughout the lifetime of the patient and all too often will be transferred to the children. Microscopic examination shows an endothrix pattern with associated air bubbles within the hair shaft. There is a distinct fluorescence under ultra violet radiation, but more green in color and less brilliant than in *Microsporum* infections.

Tinea Barbae

Ringworm of the beard is rare except in a few geographic areas It closely resembles the inflammatory forms of tinea capitis and is usually due to *T. mentagrophytes, T. verrucosum, T. violaceum* or *T. tonsurans.* There is frequently associated bacterial infection, and treatment is difficult.

Tinea Corporis

Any of the previously mentioned fungi can cause infection of the non-hairy skin, indeed, tinea capitis is frequently combined with tinea corporis caused by the same organism. Clinically, rounded scaly placques occur (usually few in number when compared with the exanthemata) which present an inflamed, slightly elevated vesicular border. These areas expand peripherally and leave the central portions clear except for scaling. Not infrequently a new papule starts in the center and enlarges; sometimes several such concentric circles can be seen, as in a target. Itching is usually prominent.

Microscopic study of scales from the active border, prepared by clearing with heat and potassium hydroxide solution, reveal hyphal threads of fungi, tending at times to break by way of cross walls into box-shaped arthrospores.

In addition to the above there are two rare specialized types of tinea corporis. One, called *tinea imbricata* (tokelau) occurs principally in the Pacific islands, southern Asia and Africa and South America It is caused by *Trichophyton concentricum*. Clinically it exhibits mild inflammation, severe scaling and polycyclic patches by coalescence, at times resembling itchyosis. The second rarity is the equivalent of kerion of the scalp, a tumid, boggy, painful, deeply involved infection usually present in one or a very few areas on the extremities *Trichophyton verrucosum* or *T. mentagrophytes* are usually causative, and the organism is acquired by contact with infected cattle, rabbits, rats or mice.

Tinea Cruris

Ringworm of the groins, gluteal cleft, axillae, and submammary and umbilical areas is quite obviously

strongly influenced by the tendency for these regions to sweat abnormally. It may at times be differentiated from intertrigo or the more common bacterial infections by asymmetry, and by raised, vesicular, actively progressing rounded borders extending beyond the edges of the contiguous areas. The commonest causative organisms are *Candida albicans*, *Trichophyton mentagrophytes* and *Epidermophyton floccosum*. The first of these has a tendency to form peculiar satellite areas which have a scale at their periphery rather than in the usual central location. The preference of the dermatophytes for keratin is revealed by the relative freedom from involvement of the scrotum, penis and labia minora, except when *Candida* is the infecting organism (moniliasis), another point of differentiation from intertrigo and bacterial infections.

Tinea of the Feet and Hands

The interdigital areas in hyperhidrotic feet very frequently become involved in an acute, vesicular denuding process very similar to tinea cruris, which may also arise in or spread to the soles or dorsal areas as well. A large percentage of persons have at least a little of this trouble at times in hot weather The infection can burrow beneath the thick keratin layer of the sole until it is completely denuded Secondary bacterial contamination is common The hands may be involved in a similar process, but much less frequently, and almost never unless the feet are also involved This form, although clinically severe, usually responds rather quickly to treatment. The causative fungi are usually *T. mentagrophytes* and less frequently, *Epidermophyton floccosum*

A strongly contrasting type is that which is caused by *Trichophyton rubrum*, an extremely chronic, mildly erythematous, scaling eruption limited principally to the

soles and palms. It is surprising (and at this time totally unexplainable) to find frequently that one hand or foot can remain involved for months or years without its mate becoming infected. This form is so notoriously resistant to therapy that it is not curable in perhaps three fourths of the cases. When this infection is severe, it is often accompanied by extensive tinea corporis or cruris caused by the same organism, in which locations it is usually more easily cleared.

Tinea of the Nails

Fungous infection of the nails may occur alone, but is frequently associated with skin involvement, usually of the feet. The commonest type begins at the tip of the nail and gradually moves proximally, causing discoloration, loss of luster and the accumulation of a dry sawdust-like detritus between the nail and its bed, eventually filling the entire space to the base The nail becomes fragmented peripherally. The causative organisms are usually *Trichophyton rubrum* or *mentagrophytes;* occasionally *Epidermophyton* is recovered

A different clinical picture is produced by *Candida albicans* (moniliasis). There is usually an associated paronychia and onycholysis, but detritus mentioned above does not accumulate.

Miscellaneous Skin Mycoses

There are four fungus infections which always remain entirely limited to the keratin of the skin or hair, and hence never produce inflammation in any degree whatever The commonest of these is tinea versicolor, caused by a fungus which can seldom be cultured artificially, *Malassezia furfur* The upper portion of the trunk and extremities are the favored areas, and multitudes of macular scaly spots, from 0.5 to 2 centimeters in diameter are

seen, eventually coalescing into polycyclic areas. The involved portions may either be lighter or darker than the surrounding normal skin, and fluoresce under filtered ultraviolet radiation. There are never any subjective symptoms, and the disorder is entirely of visual significance.

Erythrasma selects certain individuals and produces a macular hyperpigmentation with a small degree of scaling in the groins, axillae or submammary regions. There are no subjective symptoms and no erythema is produced. The cause is *Nocardia minutissima,* which also resists culture.

Trichomycosis axillaris produces nodules or concretions on the hairs of the axillae or groins, either yellow, red or black in color. A fungus, *Nocardia tenuis* has been considered to be the cause, but a recent report considers the organism to be bacterial.

Piedra produces stony concretions along the shafts of scalp hairs. It is usually attributed to two different fungi, *Piedraia hortai,* a black type, and *Trichosporon beigelii,* a white form. There is some doubt about the latter entity.

Chromomycosis is caused by one of several strains of fungi, sometimes given species status, and known as *Cladosporium, Hormodendrum* or *Phialophora.* Everting, warty areas are produced, and penetration beneath the basal layer occurs. This is not strictly a dermatophytosis, but nevertheless cannot be called a "deep mycosis." The feet and legs are most commonly involved.

Rhinosporidiosis is also intermediate between the superficial and the deep mycosis. It is caused by *Rhinosporidium seeberi*, acquired by contact of the face with the bottoms of rivers Friable masses of granulation tissue are produced within the nostrils, mouth or eyes.

Chapter 21

DERMATOPHYTOSIS—IMMUNOLOGIC AND ALLERGIC MANIFESTATIONS

Resistance to Infection

Most, if not all, human beings become infected at some time or other by dermatophytes; frequently this process is highly repetitive throughout their lives. During these episodes the natural keratinization process is continually producing scales, and hair and nail fragments which are shed into the environment, and which are loaded with viable fungus spores. Also, many of the animals commonly kept as household pets are capable of acquiring fungous infections and transmitting them to their masters. In addition some species of dermatophytes are apparently widely distributed over the globe in the soil, where they grow saprophytically. It is thus obvious that no one can live without frequently and repeatedly having his skin brought into contact with these pathogenic organisms. The degree of virulence or pathogenic ability possessed by these fungi is subject to *some* variation but not . . any wide range.

In the light of these facts it would *seem* at first glan to be inevitable that almost all human beings at al all times would be actually infected with many , these infections. Of course, such is *not the case*, there are many factors which are known (and m many others which are not yet known) which

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generation of urea. The thick soled, heavy unventilated shoes long considered stylish by men, account for a large part of their increased susceptibility to infection over women who wear mere "soles and straps." Obesity causes deep folds between the buttocks and in the groins and axillae which easily become macerated; the situation is then usually worsened by the wearing of tight girdles.

Immunologic factors may be either *natural* or *acquired.*

Natural Immunity

Although difficult to prove statistically, and contrary to the results to be expected from much animal experimentation, it seems evident that a high proportion of human beings are naturally resistant to most dermatophytes. For example, even in communities where little or no effective measures are taken to combat the epidemic form of tinea capitis, the *percentage* of children who become infected *always remains small,* and the epidemic eventually reaches a "leveling off" stage. It is difficult to accept the thesis that this occurs wholly because of mechanical or virulence factors such as those previously mentioned, leaving a strong inference that susceptibility is by no means universal.

Another example is furnished by studies of human infection by *Trichophyton rubrum* In a series of 61 persons so infected continuously during from 1 to 20 years, Wilson, Levitt and Plunkett found that not one spouse had acquired the disease, even though obviously living in a sea of spores of the organism for such long periods. From this observation no accurate percentage can be derived, but it is probably correct to state that not more than one in a hundred persons is *capable* of becoming infected by this fungus It appears highly unlikely

conceivable variations to occur in the range of *individual susceptibility* to infection. Aside from the previously mentioned variations in virulence of the causative organisms, these factors may be either mechanical or immunologic.

Mechanical factors are obviously very important, since they often act as adequate barriers to the acquisition of a dermatophytosis. Frequent bathing with soap and water removes a large proportion of those infectious elements which fall upon the skin before they become pathogenically established, and also diminishes the quantity of organic residues from perspiration, some of which at least can serve to feed fungi. Persons whose skin naturally is *thin, with little thickness to the keratin layer which fungi love*, are more difficult to infect. A dry type of skin which sheds its keratin rapidly is more resistant than one which *is moist and oily. Occupations necessitating frequent* wetting of the skin, especially with liquids containing nutritive food elements, promote infection.

The keratin in human hair is apparently too dry and inert to attract and support fungi except for those portions still within the follicle and for a very few millimeters beyond its orifice. Hence, long-haired girls acquire tinea capitis much less frequently than short-haired boys, since those oragnisms which fall upon their heads less frequently reach the scalp. This factor is even noticeable in boys, for the clipped, comparatively short-haired nape is much the favored initial spot for the infection. Although there are some fatty acids in sweat which possess fungistatic abilities, excessive perspiration actually *promotes* infection, especially when occluded from adequate evaporation as in the feet, axillae, groins, and submammary folds. Perhaps in these areas the fungistatic fatty acids are somewhat neutralized by ammonia resulting from the bacterial de-

generation of urea. The thick soled, heavy unventilated shoes long considered stylish by men, account for a large part of their increased susceptibility to infection over women who wear mere "soles and straps." Obesity causes deep folds between the buttocks and in the groins and axillae which easily become macerated; the situation is then usually worsened by the wearing of tight girdles.

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that such a degree of resistance can be explained by any mechanism of specific acquired immunity due to previous acquisition of the disease in an unrecalled form. This resistance thus seems to be of the *natural* variety, leading to the conclusion that those who do become infected either *lack* this *normal factor*, or *possess* some *abnormal characteristic* making them peculiarly susceptible. That this abnormality may even be genetically transmitted, appears possible also from the report of Wilson, Levitt and Plunkett who observed that in the families of the 61 patients there were two *children* (blood relatives) who had acquired the infection even while the *spouses* had not.

Favus, caused by *T. schoenleini* frequently persists throughout a person's lifetime and often becomes transmitted through several generations within the family. If any large percentage of persons were susceptible to such an extremely persistent disease, it would seem inevitable that it would become very common. Here again an inherited defect in natural resistance seems a likely explanation. Similarly, certain persons seem to attract *Malassezia furfur* infection almost magnetically, whereas most are apparently naturally immune. This, also exhibits certain familial concentrations suggesting that a hereditary factor may be of controlling importance.

Perhaps this "natural immunity" is simply a characteristic of complete general good health, and susceptibility occurs only as an accompaniment of abnormality, either as an inherited factor or as the result of disease. For example, diabetes predisposes to moniliasis, as does vitamin B deficiency and the oral administration of certain antibiotics. Parathyroid disease may make a person susceptible throughout life to this infection.

It is at once apparent that at least as much effort should be expended in attempting to learn the nature of

these defects in natural resistance to disease as in searching for chemicals capable of curing the infections by killing the offending organisms. In fact, fungicides alone probably can only be helpful temporarily in many disorders, because the still susceptible person will soon become reinfected after its cessation.

In contrast to the above, there are some fungous infections to which most (or perhaps all) persons are probably originally susceptible, such as tinea pedis due to *T. mentagrophytes.* Large numbers of people possess this infection continuously over many years, others acquire it frequently and clear it relatively quickly each time, seemingly because of a resistance factor easily developed or regenerated by the infection but which disappears, or at least declines below a totally effective level in the interims. Some of these persons eventually appear to become persistently immune.

A similar fluctuation or alternation in immunity and susceptibility is seen in the ordinary type of tinea corporis, but in this case exhibited as a temporary variation in tissue resistance. Here, an actively advancing border leaves behind an area which clears itself of the infection and cannot be reinfected for a time, but soon loses this resistance allowing a new center to start which then expands and clears again centrally, resulting in a target of concentric rings, a picture responsible for the acquisition of the name of "ringworm."

Acquired Immunity

When tinea capitis is associated with marked inflammation, as in kerion Celsi, the prognosis is good The infection seems to "burn itself out" and the patient almost never becomes infected again subsequently This observation seems to indicate the development of a permanent

immunity of a definite specific and acquired type. Associated with this in a very significant manner is the development of an *allergic hypersensitivity which will now be discussed.*

TRICHOPHYTIN REACTIONS. From cultures of dermatophytes of any species, extracts called "trichophytins" can be made, which are of value in certain testing procedures and occasionally in the treatment of disease. It has usually been concluded that the product is identical no matter which species of organism is utilized (except for *Candida*). In other words, trichophytin is "group specific" rather than "species specific."

In sensitive individuals the intracutaneous injection of trichophytin produces either an immediate wheal (urticarial) response or a delayed, tuberculin type of reaction reaching its peak in about 48 hours.

The delayed, tuberculin type of reaction has received the larger amount of attention, and is what is usually meant by the statement "positive reaction to trichophytin." *As stated before, this reactivity develops in the* skin in general of patients with tinea capitis when kerion supervenes, and is hence closely associated with recovery. This sensitivity however endures long after cure has been obtained, perhaps for the life of the individual. Since such patients rarely become reinfected, at first glance it is tempting to accept as fact that this reactivity is the means by which immunity is implemented. In coccidioidomycosis the parallelism is even more closely documented, but it is still not possible to connect the two definitely. In dermatophytosis there are many more gaps in our knowledge, and at present it must be considered as conjectural.

Hypersensitivity to trichophytin is also present in marked degree in many patients possessing other dermatophytoses, especially of the acute, inflammatory, vesicular

types such as tinea pedis or cruris. In fact it seems certain that a considerable proportion of the acute inflammation usually present in these disorders occurs as a result of this exquisite allergic hypersensitivity to the products of the invading fungus, which must be closely similar or identical to trichophytin. The vesiculation which is produced by this mechanism is obviously of assistance in clearing the body of the infection, since it separates the keratin and pre-keratin layers almost anatomically from the rest of the epidermis, and leaves no keratin to support further fungal growth. Here, however, even though this type of disease is usually easily cleared, indicating the value of such a "specific acquired resistance factor," these patients can be subsequently reinfected over and over again, as soon as new keratin is produced, even though the hypersensitivity persists apparently unchanged throughout the years. Such an immunity as this is obviously not perfect nor complete.

Dermatophytid Reactions Frequently in association with a dermatophytosis in a localized area such as feet, groins, or scalp, an erythematous, papulo-vesicular eruption develops at a distance from the original focus This may be localized or generalized, mild or severe. It is caused by the dissemination from the actively infected focus by way of the blood stream of fungous elements or products. In such individuals, hypersensitivity to trichophytin of the delayed tuberculin type is the rule, in fact it is a requisite for accepting the eruption as a trichophytid. Although this hypersensitivity probably helps the patient to "throw off" the actual infection, it is frequently much more uncomfortable than the original disease. All active fungicidal treatment must be abandoned to reduce the absorption of dead fungous derivatives and thereby ameliorate the allergic reaction, and gentle anti-

eczematous measures substituted, together with temporary support by anti-allergic drugs such as antihistaminics and steroid compounds. Specific desensitization and maintenance in that desensitized state by cautiously repeated injections of trichophytin in appropriately increasing amounts may be necessary.

IMMEDIATE WHEAL REACTIONS to the intracutaneous injection of trichophytin are commonly encountered and are considered by many authors as equally specific and meaningful as the delayed tuberculin type. It is usually advocated that both of these reactivities are closely associated, in fact different stages of the same process. It is claimed that when hypersensitivity develops, the *first* manifestation is the ability to react with the delayed, tuberculin type of response. This is considered to be due to antibodies which are developed and fixed to the cells of the skin. This type of reactivity cannot be transferred through serum to another individual and does not pass the placenta. It is therefore concluded that the antibodies are not present in the circulating blood, at least in the serum. It is believed that later as the process of hypersensitivity develops more fully these antibodies become detached from the cells and thereafter circulate with the blood, now being transferable. They now confer upon the individual the power to react to the intracutaneous injection of trichophytin by the formation of the "immediate wheal" response

This is an appealing thesis, and conforms to much investigational work with allergens derived from foods, danders, protozoa, animal parasites, rickettsiae, viruses, and bacteria. However, these substances are uniformly of a protein nature, whereas trichophytin is almost a pure polysaccharide It has already been pointed out in relation to coccidioidin (see page 52) that it is not neces-

sarily correct to expect such dissimilar substances to react identically. There are, indeed, several discrepancies in this concept which need clarification.

Trichophytin (even as coccidioidin and the other antigens derived from fungi) is not completely antigenic in its own right. In other words, repeated injection of trichophytin into normal persons cannot induce the ability to react to its subsequent intracutaneous inoculation by the production of the delayed tuberculin type of response. It is possible however to induce the immediate wheal reactivity. Since the former is considered to be the *first* of the two "stages" of the development of hypersensitivity how can the "immediate wheal" type occur without being preceded by it? Trichophytin is the only product derivable from dermatophytes growing in culture and is admittedly not completely antigenic. It is thus obvious that fungi growing on the human body in an actual infection must produce some *completely* antigenic product differing from trichophytin, since hypersensitivity *is* produced under *those* circumstances. Since the entire cutaneous surface becomes hypersensitive and reactive at the same time, it is obvious that this antigenic substance must circulate in the blood stream Thence, it must be extracted by those cells of the body which produce antibodies, a function usually attributed to the reticuloendothelial system. The skin is relatively very poorly supplied with reticuloendothelial elements of the type usually credited with this ability. Are we to assume that antibodies are formed in the spleen, liver, lymph nodes and other tissues rich in reticuloendothelial elements and then transported quantitatively to skin cells to become fixed there suddenly? Must these antibodies not therefore circulate in the blood to get to the skin? If so why do we not observe the "immediate wheal" reaction to intracutaneous

testing *first,* since this is the characteristic reaction to "circulating" antibodies? Why must we attribute to Nature an illogical process of fixing antibodies to the skin at first and only later releasing them to "circulate"? While this may not appear to be entirely out of order in assisting the body to resist an invasion of fungi into the *skin itself, such as with dermatophytosis, it is utterly in*comprehensible as a method of resisting a pulmonary infection, as in the deep mycoses, where effective immunologic resistance *does* develop, and is associated with skin test reactivity of the delayed, tuberculin type, ("fixed antibodies to skin") but never consistently with the urticarial, immediate wheal reactivity ("circulating antibodies"). *It* is easier to believe that the antibodies involved in the delayed tuberculin type of response *do* circulate when necessary, and that our tests have simply not as yet revealed them there.

The possibilities here may be seen at once by asking how likely would be the chance of obtaining a fire-engine in a routine sampling of the traffic arteries of a city, and considering in contrast what happens when a fire is reported. They may not be "fixed" to the skin at all, but called there from elsewhere in response to the injection *of the antigen.**

At present, it seems better to consider both reactions as separate entities, not stages of the same process convertible one into the other. The delayed tuberculin type of reactivity may be associated with immunity in some

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* [illegible] (that induced by [illegible] skin, Haxthausen showed in two pairs of identical twins, that the antibodies were *not* fixed to the skin. Skin from the sensitized partner lost its reactivity when transferred to the nonsensitized partner, and that from *the latter acquired reactivity upon transfer to the former*

way; the immediate flare reaction is probably not. This parallels experience in coccidiodomycosis and other deep mycoses.

In line with this opinion it has been pointed out that persons infected chronically with *Trichophyton rubrum* react to trichophytin much more frequently with the immediate wheal than with the delayed tuberculin response. It must be considered significant that they also usually do not succeed in resisting the disease *at all;* even the erythema which accompanies their infection is consistently *minimal*, and the disease persists for many years. Vesicular reactions, which could help the body to throw off the infection are uncommon, and trichophytid reactions are very rare.

Attempts have been made to render trichophytin completely antigenic by attaching it to other substances, such as *M. tuberculosis* lipoid or protein-like materials None has succeeded in inducing the tuberculin type of sensitization subsequently, nor has any degree of immunity been observably conferred Attempts have been made to induce the delayed type of trichophytin reactivity in persons infected with *T. rubrum* in the typical highly resistant form, by infecting them with *T. mentagrophytes,* hoping to enhance the acquisition of specific immunity; as yet no notable success has been attained.

Chapter 22

DERMATOPHYTOSIS—THERAPY

THE PRESENTLY available methods of treatment for the dermatomycoses are far from satisfactory. This seem surprising in view of the fact that these infections have been studied for nearly 120 years, and that at all times there has been a plethora of clinical material. One reason is that the disease practically never becomes sufficiently severe to warrant being classed as more than a nuisance, although sometimes a major one. As a corollary to this, only a tiny fraction of the scientific brain power of the world was attracted to mycology during its first century, and those few expert mycologists who did develop were severely handicapped financially in obtaining assistance in their studies. With the last two decades witnessing more and more major victories in other fields of medicine, mycology has been increasingly attracting the attention of competent scientists, so that the future appears promising.

However, there is one factor which seems to have been insufficiently emphasized in the past, which has probably done more to slow our progress than any other. This is the failure to realize that there is a highly important mechanical characteristic to the dermatomycoses in the manner in which these organisms behave. They thrive in keratin, and if given the correct circumstances and only a little time, they succeed in penetrating as deeply into the body as does the keratin. This is relatively unimportant except where keratin is present in thickness or depth, such

as in nails, hair follicles or palms and soles, but in these regions it consistently defeats our best attempts at medication. The plain truth of the matter is that as yet no chemical or method has been devised which can extend its fungicidal effects as deeply into these keratinized structures as the fungi regularly penetrate. Thus, even chemicals demonstrably capable of killing specific fungi in high dilutions (less than one part per million) have failed miserably to cure or control certain forms of tinea of the scalp, nails or soles. In fact, many students of these disorders believe that the external application of chemical fungicides to the affected parts exerts no beneficial influence whatever.

In the opinion of this author this is not entirely true, because of a phenomenon called the "epidermal effluvial current" This is not a new concept, but it certainly needs more emphasis than it has previously received. In man and in animals, the epidermis proliferates continuously and pushes the epidermal cells thus produced toward the external surface to be shed eventually as dried keratin squames. This process goes on just as productively within the hair follicles as it does on the flat surfaces of the skin. The hair itself is produced by this activity on the part of the epidermal cells of its root at the papilla, and it is, of course, well recognized that it grows outward The basal cells of the external root sheath are equally productive; however, they do not contribute their keratinized product to the hairs, but to the inner root sheath, whose two layers are known as the sheaths of Huxley and Henle. It is not so well recognized that this keratin material also grows outward at the same rate as does the hair and appears at the mouth of the follicle as a ring of keratin surrounding it before being shed Thus, not only the hair itself but all of the keratin and its precursors which are

produced within the follicle are carried outward at the same rate, and there is no sliding of one layer abruptly over another. *This process I think of as the "epidermal effluvial current."*

To succeed in parasitizing a hair, a fungus must be able to grow downward into the follicle at a rate more rapid than the rate of this "epidermal effluvial current." Also, since a fungus does not ordinarily penetrate *beyond* the confines of the epidermis, unless fortified by bacterial allies or by allergic hypersensitivity, it will always be contained *entirely* within structures which are moving toward the exterior at this same rate. Hence, to maintain *its parasitism of the follicle the fungus must continuously* grow downward into the follicle at a rate at least equal to or exceeding the "epidermal effluvial current" In common language, it must continuously swim upstream to avoid being carried out to sea and lost. If the fungus falls behind sufficiently in its rate of growth, the follicle will clear itself *automatically* of the infection. This is true regardless of whether or not the hair shaft itself is discharged as the result of the natural cycle or becomes epilated by other means, for the epidermis of the root sheath maintains this outward current at the same rate even when the hair shaft is not present, unless it is in a totally resting state, in which case no keratin is being produced nor is there any present to support the growth of the fungus

I believe this concept explains many otherwise puzzling aspects of the therapy of tinea capitis. Cure will occur if the rate of hair growth becomes sufficiently increased or the ability of the fungus to grow becomes sufficiently slow The rapidity of growth of the advancing filaments at the edge of a colony of fungus depends to a significant degree upon the amount of *fungus present for*

its support in the more central portions and upon its degree of health. There are probably many factors which can cause sufficient variations in the health status of the fungus to slow its growth enough to allow a cure to occur. Some of these factors are undoubtedly inherent in the fungus itself, and the cure appears to be spontaneous even in the absence of any immunologic resistance on the part of the host. Closely clipping the scalp and cleansing it frequently and vigorously with soap and water is undoubtedly sufficient in many instances to tip the balance in favor of cure. Also, it is not necessary for a fungicide to be capable of penetrating to the *depths* of the follicles and killing *all* of the fungi contained therein to be credited with *contributing* to the achievement of a cure. It is only necessary for it to maintain sufficient fungistatic effect upon the surface, and to a certain depth perhaps, to slow down the penetration rate of the parasite so that its rate of growth downward into the follicle finally becomes less than the rate of the "epidermal effluvial current." It also can serve by preventing the infection of *new* follicles. If this effect is maintained long enough over all the scalp, all of the follicles will clear themselves The selection of an individual *fungicide* thus becomes much less important than the length of time *some* such agent is *continuously* applied Because of peculiar medicolegal factors, epilation by means of x-radiation is seldom employed in Southern California We have thus been forced to attempt to cure almost all cases of tinea capitis by externally applied medication continued steadily for many months. Under these circumstances, we have observed a much higher *eventual* cure rate than reported elsewhere, of even such resistant infections as those caused by *M. audouini*, *T. tonsurans*, and even *favus*.

This same mechanism is active in identical fashion in nails, except that it is a great deal slower and is exerted on one side of a concave surface instead of the entire periphery of a circle, as in a hair. I believe that whenever fungous infections of nails become cured, *it is the "epidermal effluvial current"* which is almost entirely responsible. Fungicides are relatively ineffective and can only contribute by attenuating the virulence of the mass of fungus with which they can be brought into contact, *thereby slowing down the penetration rate of the deep* advancing filaments.

In general, effective fungicides will prove beneficial only for disorders actually caused by fungi and are likely to irritate the remainder. It is thus important *to confirm the clinical diagnosis of fungous infection* wherever possible by irrefutable laboratory means. The direct microscopic examination for fungi of specimens of diseased tissue is easy, and should be considered almost mandatory. Cultural studies are equally necessary in certain types of *infection, and should be routinely employed in all forms* wherever facilities are easily accessible.

In addition to determining which type of infection is present, it must be emphasized that attention must be given also to its *stage*, in order that a therapy may be selected *which the tissues can tolerate.* Strong medications should never be applied to acutely inflamed areas nor to those inherently subject to irritation such as eyelids, anus, or genitalia. Whenever there is doubt, *the weaker* remedy will usually prove to be the better choice.

Secondary infection by pyogenic bacteria is frequently superimposed upon fungous infections, and must ordinarily take precedence in the selection of treatment. Also, there are many times when therapy *must be modified be-*

cause of the development of allergic hypersensitization to the infecting fungus or to its products

Tinea Capitis

Once the diagnosis of ringworm of the scalp has been made, it is strongly advisable to clip the hair completely, so that medication may be more easily applied and with much less waste, once or twice daily, and the scalp can be easily shampooed with soap and water every day or two. Infected hair fragments are thus less likely to remain in contact with uninvolved areas long enough to start new foci. A closely fitted cap fastened beneath the chin and made of material impervious to infected hair fragments can be worn to prevent scattering them about to result possibly in infecting other persons.

NONRESISTANT INFECTIONS, most commonly caused by *M. canis*, *M. fulvum* or *T. mentagrophytes*, must be guided therapeutically in accord with the degree of inflammation present. In its most pronounced form, known as "kerion," this inflammation is severe, causing large, boggy, purulent, painfully swollen areas, frequently associated with systemic toxicity. In this stage only the mildest of wet dressings or soaks will be tolerated, such as saturated solution of boric acid or diluted Zephiran.® When severe, (and especially if there is systemic reaction such as fever) the systemic administration of penicillin or a wide spectrum antibacterial antibiotic is indicated. In this stage strong fungicides should *never* be used because they intensify the inflammation, by the same token they are almost uniformly *unnecessary* because the infected hairs are caused to be shed from the follicles by the effects of the inflammation and, a tendency towards a spontaneous cure results, largely from specific immunologic causes. As this process subsides however, close observation is in-

dicated, since some more resistant patches may remain. Kerion can occur in infections due to *any* of the previously named organisms, even at times in those which at first were classed as "resistant"; when this happens, of course, they lose this designation.

For cases exhibiting lesser degrees of inflammation, ever stronger remedies will be needed, and will be better tolerated, although the "kerion" stage may develop at any time and indicate their discontinuance. The presence of any considerable degree of inflammation calls for compresses or soaks of Alibour solution (copper sulfate 0.02% and zinc sulfate 0.06%). Somewhat less inflamed cases can be given mild fungicides such as ammoniated mercury ointment 5%, or 5% sulfur ointment with or without 2 to 3% of salicylic acid. Milder degrees of inflammation call for more strongly antifungal remedies, many of which are available as proprietary compounds such as Salinidol® (5% salicyl-anilide), Asterol® 5% (not to be used for children under 5 years of age because of possible toxicity), Salundek® (salicylanilide and undecylenic acid) Decupryl® (undecylenic acid and a copper compound) or Verdefan®. An advantage possessed by some of these ointments is that the base is carbowax, a water soluble grease, which facilitates the shampoo Iodine 2% in the same base is also good as is a mild form of Whitfield's ointment (5% salicylic acid 3% benzoic acid).

It has long been evident that on medication yet devised can penetrate as deeply into the hair follicles as do the infecting fungi, hence no one of the above medications can be said to be *reliably* effective, and there is little indication that any one of them is superior. Since fungi seem able to develop resistance to any particular medication after a time, it is advisable to change the prescription every 4 to 6 weeks, no matter which one is chosen at first Any

thing which promotes the shedding of infected hairs assists the cure, and many operators believe that manual extraction of fluorescent hairs by forceps (or massively by using strips of adhesive tape) under the guidance of the dark light is helpful. Inflammation itself causes much shedding of hairs, and kerion does so quite completely, hence the ease of cure when these are present. Subsequently, continuous medication prevents reinfection of those follicles whose hairs have once been shed and thus contributes to progress.

Resistant Infections. In most geographic regions, a patient possessing proved ringworm of the scalp, and presenting no inflammation (especially if it is shown culturally to be due to one of the fungi of resistant characteristics), should be given the benefit of complete temporary epilation of the scalp hairs by the administration of x-radiation by an experienced operator. Medicolegal considerations deny this in some localities, and lack of available x-ray facilities or experienced personnel in others This method is also not usually employed below the age of 3 years, nor in the *Microsporum* infections when the patient is approaching puberty X-ray epilation must be very carefully performed or permanent alopecia may result, hence a competent dermatologist or radiologist should be in charge. After the epilating dose is applied, only mild medicaments should be used, ammoniated mercury (3%) is usually chosen and is applied daily until the hair has all been shed.

When x-ray epilation cannot be employed for any of the above reasons, local medication will provide a reasonable chance of cure, but only if carried out rigidly and continuously over a period of from 4 to 6 months or more. The stronger medicaments previously listed should be utilized and changed occasionally. X-ray therapy may

also be delayed or dispensed with entirely at times when the involved area is small and well localized.

CRITERIA OF CURE. Patients with tinea capitis should be closely supervised for several weeks after the last indication of persistent infection has disappeared. In the fluorescent types, the dark light is helpful, and avoids much culture work. When fluorescence is no longer seen, then cultures should be taken from at least two previously involved areas on at least three occasions at 2 week intervals, and shown to be negative before cure is presumed Relapses are frequent if this procedure is not followed, and there are no more disgruntled parents than those who have to begin all over again with the complicated treatment schedule some months later, perhaps also with several other children who have become infected in the interim!

Epidemiologic rules vary according to locality and type of infection, but there is little danger of transmission if the cap is always worn, ointment is always present on the scalp, and bodily contact between children prevented. Especially safe in this regard are the inflammatory types of animal origin. However, infected children are usually excluded from theaters and barber shops. Kittens and puppies must be suspected as original foci, and examined. When cases of resistant tinea capitis are discovered, the fact should be given wide publicity in the locality, so that all possible contacts may be examined soon and any resulting infections discovered while yet mild in extent.

Tinea Barbae (Ringworm of the Beard)

As with tinea capitis, various degrees of inflammation are encountered. *T mentagrophytes* and *T. verrucosum* (from cattle, rabbits, mice or rats) cause an inflammatory type, frequently duplicating the kerion stage of tinea

capitis, while *T. rubrum*, *T. tonsurans* and *T. violaceum* cause a comparatively noninflammatory form. As with the scalp disorders, inflammation usually means less resistance to treatment, but this is not so reliable here.

Marked inflammation calls for mild wet dressings with boric acid solution or diluted Zephiran®. Less inflamed areas will tolerate Alibour's copper and zinc sulfate solution and mild fungicides such as 5% ammoniated mercury or sulfur and salicylic acid ointment (5% and 3%). Frequently it will be necessary to combat secondary bacterial infection before beginning specific antifungal therapy. In the post inflammatory stage or when dealing with a noninflammatory form, stronger antifungal measures are necessary, including those recommended above for tinea capitis. The beard may be clipped, but shaving should be avoided since it tends to spread the infection. Fractional x-radiation is beneficial, and occasionally, if the disease is recalcitrant, it may be necessary to use a dose capable of causing temporary epilation, as with tinea capitis.

Tinea Corporis (Ringworm of the Glabrous Skin)

Infection of the non-hairy skin by fungi may occur alone or in combination with scalp disease; and whenever one of these is discovered, the other should be suspected. When both are present, it is to be expected that the skin lesions will respond to treatment within 2 or 3 weeks while the scalp often requires several months. This is because of the lack in the former of the deeply implanted hair follicles, which furnish the pathway for deep fungus penetration in the scalp.

In childhood, members of the *Microsporum* group of fungi are mostly responsible, in adults the commonest are *T. mentagrophytes* and *T. rubrum*. There is little clinical

difference between infections caused by the different varieties of fungi, but there are different grades of inflammation, which must be considered in the selection of therapy. It cannot be over emphasized that many nonfungal skin disorders can produce the ringed lesions so firmly fixed in the minds of clinicians as diagnostic of ringworm. Direct microscopic examinaton will confirm or deny the diagnosis.

When inflammation is severe, wet dressings are indicated, using boric acid solution or Alibour water (see above). If secondary bacterial infection is present it must be treated with local antibiotics. As the inflammation subsides, mild fungicides such as sulfur-salicylic acid ointment (5% and 3%) can be used. Diluted tincture of iodine (1%) is good. In this stage practically any antifungal preparation will achieve a cure within two weeks, if irritation does not develop. To prevent recurrence, an attempt should be made to discover and eliminate the source of the infection. To facilitate this, cultural studies will frequently discriminate between human and animal sources

Tinea Cruris (Ringworm of the Groins)

Almost without exception strong materials should *never be used in intertriginous areas such as the groins,* intergluteal cleft, submammary creases and axillae. Astringent antiseptic wet dressings, such as mentioned above *but usually only half or one third of the usual full strength,* are best in the inflammatory stages These should not be applied continuously, but intermittently, allowing plenty of time for the areas to become dry between treatments; at most they should be used 20 minutes out of every hour, and usually such a session three or four times daily will suffice. Copious amounts of plain talcum powder

should be applied between sessions and as little clothing worn as possible; allowing air to assist in maintaining dryness. Avoid medicated powders, or those containing starch which can feed fungi or bacteria when wet. A mild sulfur (3%) salicylic acid (2%) preparation in a vanishing cream or carbowax base (water soluble) is serviceable, while a greasy ointment is likely to be poorly tolerated and to cause folliculitis in hairy regions. If this material is applied thinly enough, the talc can also be continued to promote dryness. Calamine lotion containing 3% resorcinol is frequently helpful. When inflammation subsides, antifungal proprietary preparations can be used with benefit, but are better diluted with a bland base to one-half or one-third their usual strength and applied sparingly to avoid irritation. It is often advisable to avoid allowing such materials to touch delicate structures such as the scrotum, where a suspensory is useful.

Moniliasis dictates special alterations in the above regimen. First, a search for one of the predisposing causes for this infection should be made and appropriate therapy begun (diabetes, vitamin deficiencies, glandular disturbances, recent administration of broad spectrum antibiotics, etc.). In the acute stage, gentian violet in 1% aqueous solution is a useful adjunct to the wet compresses, even though it is known that the monilial fungus (*Candida albicans*) frequently is able to grow in the presence of this medication. At this writing, an antibiotic, "nystatin," in ointment form seems destined to achieve a reputation for effectiveness against moniliasis, although not for infections caused by other fungi. Moniliasis is prone to recur especially if one of the predisposing causes mentioned above remains uncorrected. Fractional x-radiation expertly administered is a helpful adjunct.

Tinea of Feet and Hands

As with the other dermatomycoses described above, ringworm of the hands and feet varies widely in the degree of inflammation produced. In the most acutely inflammatory stage vesiculation is severe, and large areas become denuded by coalescence of vesicles into bullae which rupture. Secondary bacterial infection is common, and very frequently of serious importance. The development of allergic hypersensitization is often evident (the so-called "dermatophytid" or "id"), and requires particular attention (see below). In any of these stages, treatment must begin with soaks or wet dressings of mild medicaments such as boric acid (saturated solution) or liquor aluminum acetate (Burow's solution) 1 to 30 to 1 to 10. Secondary bacterial infection requires the substitution for the above of potassium permanganate (1 to 10,000 to 1 to 2,000,) or Alibour's copper and zinc sulfate solution (0.02% and 0.06% respectively.) Such wet dressings should be used intermittently for only 20 to 30 minutes, 3 to 8 times daily with periods between the sessions devoted to air drying. Bullae and large vesicles must be opened so that their burrowing tendency is stopped and so that the medicated solution can reach the tissues actually diseased. Sometimes gential violet (1% aqueous solution) is useful, especially if moniliasis is present. When bacterial infection is marked, an antibiotic ointment is useful between the sessions if applied very thinly to avoid maceration. Polysporin® or Polycin®, Terramycin®, Neosporin®, Bactitracin® and similar combinations are current favorites. Every effort should be made to promote drying of the affected areas, by using cotton pledgets to separate toes and to allow access of air and liberal applications of plain talc without starch. Only sandals or other types of shoes permitting aeration are allowed.

If inflammation is mild, or has subsided under the above regimen, wet dressings need be less frequent, but should be used at least once daily for cleanliness; the above mentioned solutions are usually to be preferred to soap and water. In the subacute stage calamine lotion containing 2 to 3% resorcinol is helpful. With further subsidence of inflammation fungicidal preparations in ointment form are recommended, such as Desenex®, Timofax®, Vioform®, Sterosan®, Asterol® or Sopronol®. Such acute or subacute types as these are frequently encountered, and are usually caused by *Trichophyton mentagrophytes* or *Epidermophyton floccosum*, although at times any of the other species named under tinea capitis will be found.

Less commonly, a dry, hyperkeratotic form of tinea affects the feet and hands, accompanied by mild inflammation only, sometimes by none at all. This form is ordinarily found to be caused by *Trichophyton rubrum*, and is notoriously difficult and frequently impossible to cure. There is considerable evidence that those patients who acquire this infection do so because they possess some inherent abnormality in their skin rendering them less able (or sometimes *totally* unable) to resist it. Many cases have been known to continue with this infection for decades, but almost never does a spouse acquire it A valuable clue as to differential diagnosis is the tendency for *T. rubrum* infection to remain unilateral (one hand or foot) In this type, the strongest of fungicides combined with keratolytics will be needed, and even then will frequently fail. Salicylic acid up to 20%, or iodine tincture up to 4% are beneficial Most of the proprietary compounds are not effective, but Asterol®, both the tincture and ointment combined is useful X-radiation in fractional amounts should be carefully added to the regimen in the early weeks. Long continuation of therapy

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is necessary. Only a fraction, perhaps 25% can be cured completely; the remainder can be taught the benefits of a fair degree of control by indefinitely prolonged medication, even when hope of cure has become dispelled.

Tinea Unguium, Onychomycosis (Ringworm of the Nails)

Fungous infection of the toe and fingernails is most commonly seen in combination with similar infection in the surrounding skin, but may persist continuously for years between intermittent episodes of the latter. This is illustrative of the extreme difficulty with which the treatment is attended. It is well to explain to the patient that the nails may be cured only if he is willing to devote considerably more time and effort to the treatment than is demanded for most other disorders. Fungi invade between the nail and the nail bed and extend their filaments deeper and deeper, frequently to the very point of origin of the nail. In the wake of this process the nail usually persists to a large extent and covers a mass of debris consisting of dirt, epithelial scales, decayed nail and fungous elements living as well as dead. It is useless to expect a medication to penetrate through this material deep enough to reach the actively progressing portion of the infecting fungus. Hence debridement must be carried out, often enough to keep up with the production of detritus (every 2 to 5 days) and as extensively as possible without producing bleeding. Even so, the base of the infection cannot be reached, and fungicides cannot penetrate deep enough. By long continued persistent medication cure can be obtained frequently, however, because the fungus mass is attenuated to the point where it cannot proliferate inward as rapidly as the nail grows outward, thus eventually losing the battle. Debridement can be carried out as an office procedure, using a motor driven dental burr or

abrasive wheel, but is time consuming and contaminates the office atmosphere with fungous spores, subsequently rendering routine cultural methods subject to contamination. There are several nonfungous disorders which can produce the same clinical picture in nails as do infecting fungi, notably psoriasis, onycholysis, and onychogryposis. These are also difficult to correct, and the treatment is different than for a fungous infection. Hence it is necessary to confirm the diagnosis at the outset if several months are not frequently to be wasted on the wrong course. The direct examination by the microscope is useful, but will be accurate only if the specimen is taken after almost complete debridement of the nail, since contaminant fungi are frequently found in the peripheral detritus which have nothing to do with the disease. Culture is often helpful in prognosis, since *Trichophyton rubrum* infections are exceedingly resistant and require selected medicaments.

It is tempting to remove the entire diseased nails by evulsion, so that medication may reach the depth, and this is often the method of choice if but one or two nails are involved. However this drastic procedure fails in a large percentage of cases because there is too much inflammation for a time afterward to permit drugs of sufficient strength to be tolerated.

Solutions of fungicides in acetone, chloroform or alcohol will usually penetrate deeper than ointments, but the best results are to be had by combining the two in succession. Iodine 2 to 4%, thymol 4%, or chrysarobin 4 to 8% are popular (CAUTION, IRRITATING TO EYES!) Asterol® 5% in tincture and ointment is helpful and seems superior in *T. rubrum* infections If not previously employed, fractional x-radiation at intervals of 1 to 2 weeks for 6 to 10 times is a beneficial adjunct, but should only be given by dermatologists on non-radiographic equip-

ment. Long and continuous therapy is essential, since intermittancy will allow the fungus new chances to penetrate. Many patients must be content with improvement of various degrees, and can usually be afforded this even when it becomes evident that complete success will not be achieved. It is a valuable semi-victory to be able to prevent the spread of the infection.

Tinea Versicolor, Pityriasis Versicolor

This infection usually responds readily to treatment, but is prone to recur, apparently because patients acquire it only by being inherently inordinately susceptible. Excessive perspiration also predisposes, and all possible efforts to combat it should be included, both during therapy and indefinitely after cure is obtained. Microscopic examination of scales from affected areas will reveal short, thick, curved hyphae and globose spores, differentiating from vitiligo and pityriasis alba. This fungus cannot be routinely cultured.

A somewhat prolonged hot bath should precede the treatment each night. Mild fungicides will suffice, a favorite is 10 to 20% sodium thiosulfate and 5 to 10% glycerin solution in water; 10 to 20% alcohol may be added if desired Almost any other fungicides will perform equally adequately, but there is little to gain thereby and the expense is increased, an important factor frequently, since the tendency is for a large portion of the skin surface to be involved.

Erythrasma

This extremely superficial disorder usually causes no symptoms, only discoloration, especially in intertriginous areas, and treatment is not mandatory. However, it usually responds easily to the same regimen recommended for tinea versicolor.

Otomycosis (Ringworm of the Ears)

Non-dermatologists are likely to label most, if not all, of the inflammatory conditions involving the external ear canals and the surrounding area as "fungous infections," but microscopic examination and cultural study seldom supports this diagnosis. Pathogenic fungi are found but rarely, and the saprophytic fungi which are recovered are present with equal frequency in normal ear canals. Seborrheic dermatitis, contact eczema due to nail polish, and neurotic excoriation are commonly found to be causative in this region. Often an actual infection is present, but not due to a fungus but to a bacteria, *Pseudomonas aeruginosa (B. pyocyaneus)*, which desponds well to polymixin B in solution (Aerosporin®, otic). Fungicides are thus not likely to be of assistance in this disorder.

Lepothrix, Trichomycosis Axillaris

This nonsymptomatic disorder, exhibiting nodular concretions on axillary or pubic hairs, is best eradicated by shaving, followed by scrupulous cleanliness and measures to avoid excessive perspiration.

Piedra, Tinea Nodosa

It is usually necessary to clip the hair closely or shave the scalp. Vigorous soap and water cleansing followed by a solution of mercuric chloride 1-2,000 is effective. Persons in close contact with the patient must be examined and treated if infected to prevent recurrence.

Chromomycosis, Chromoblastomycosis

Small and well localized areas are best treated by curettage *and desiccation For more extensive cases, the*

systemic administration of potassium iodide to the point of intolerance is advised. Recently, vitamin D_2 in maximal dosage has been recommended in addition to the iodide.

Rhinosporidiosis

Wherever possible, complete destruction of all involved tissue by cauterization or desiccation is advised. Antimony in the form of Neostibosan 0.3 gm. on alternate days for ten doses may be used.

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